Increased proangiogenic activity of mobilized CD34$^+$ progenitor cells of patients with acute ST-segment-elevation myocardial infarction: role of differential MicroRNA-378 expression

Templin, Christian ; Volkmann, Julia ; Emmert, Maximilian Y ; Mocharla, Pavani ; Müller, Maja ; Kraenkel, Nicolle ; Ghadri, Jelena-R ; Meyer, Martin ; Styp-Rekowska, Beata ; Briand, Sylvie ; Klingenberg, Roland ; Jaguszewski, Milosz ; Matter, Christian M ; Djonov, Valentin ; Mach, Francois ; Windecker, Stephan ; Hoerstrup, Simon P ; Thum, Thomas ; Lüscher, Thomas F ; Landmesser, Ulf

Abstract: OBJECTIVE Proangiogenic effects of mobilized bone marrow-derived stem/progenitor cells are essential for cardiac repair after myocardial infarction. MicroRNAs (miRNA/miR) are key regulators of angiogenesis. We investigated the differential regulation of angio-miRs, that is, miRNAs regulating neovascularization, in mobilized CD34$^+$ progenitor cells obtained from patients with an acute ST-segment-elevation myocardial infarction (STEMI) as compared with those with stable coronary artery disease or healthy subjects. APPROACH AND RESULTS CD34$^+$ progenitor cells were isolated from patients with STEMI (on day 0 and day 5), stable coronary artery disease, and healthy subjects (n=27). CD34$^+$ progenitor cells of patients with STEMI exhibited increased proangiogenic activity as compared withCD34$^+$ cells from the other groups. Using a polymerase chain reaction-based miRNA-array and real-time polymerase chain reaction validation, we identified a profound upregulation of 2 known angio-miRs, that are, miR-378 and let-7b, in CD34$^+$ cells of patients with STEMI. Especially, we demonstrate that miR-378 is a critical regulator of the proangiogenic capacity of CD34$^+$ progenitor cells and its stimulatory effects on endothelial cells in vitro and in vivo, whereas let-7b upregulation in CD34$^+$ cells failed to proof its effect on endothelial cells in vivo. CONCLUSIONS The present study demonstrates a significant upregulation of the angio-miRs miR-378 and let-7b in mobilized CD34$^+$ progenitor cells of patients with STEMI. The increased proangiogenic activity of these cells in patients with STEMI and the observation that in particular miR-378 regulates the angiogenic capacity of CD34$^+$ progenitor cells in vivo suggest that this unique miRNA expression pattern represents a novel endogenous repair mechanism activated in acute myocardial infarction.

DOI: https://doi.org/10.1161/ATVBAHA.116.308695

activity of mobilized $CD34^+$ progenitor cells of patients with acute ST-segment-elevation myocardial infarction: role of differential MicroRNA-378 expression. Arteriosclerosis, Thrombosis, and Vascular Biology, 37(2):341-349.
DOI: https://doi.org/10.1161/ATVBAHA.116.308695

Increased Proangiogenic Activity of Mobilized CD34+ Progenitor Cells of Patients With Acute ST-Segment–Elevation Myocardial Infarction

Role of Differential MicroRNA-378 Expression

Christian Templin,* Julia Volkmann,* Maximilian Y. Emmert, Pavani Mocharla, Maja Müller, Nicolle Kraenkel, Jelena-R. Ghadri, Martin Meyer, Beata Styp-Rekowska, Sylvie Briand, Roland Klingenberg, Milosz Jaguszewski, Christian M. Matter, Valentin Djonov, Francois Mach, Stephan Windecker, Simon P. Hoerstrup, Thomas Thum, Thomas F. Lüscher, Ulf Landmesser

Objective—Proangiogenic effects of mobilized bone marrow–derived stem/progenitor cells are essential for cardiac repair after myocardial infarction. MicroRNAs (miRNA/miR) are key regulators of angiogenesis. We investigated the differential regulation of angio-miRs, that is, miRNAs regulating neovascularization, in mobilized CD34+ progenitor cells obtained from patients with an acute ST-segment–elevation myocardial infarction (STEMI) as compared with those with stable coronary artery disease or healthy subjects.

Approach and Results—CD34+ progenitor cells were isolated from patients with STEMI (on day 0 and day 5), stable coronary artery disease, and healthy subjects (n=27). CD34+ progenitor cells of patients with STEMI exhibited increased proangiogenic activity as compared with CD34+ cells from the other groups. Using a polymerase chain reaction–based miRNA-array and real-time polymerase chain reaction validation, we identified a profound upregulation of 2 known angio-miRs, that are, miR-378 and let-7b, in CD34+ cells of patients with STEMI. Especially, we demonstrate that miR-378 is a critical regulator of the proangiogenic capacity of CD34+ progenitor cells and its stimulatory effects on endothelial cells in vitro and in vivo, whereas let-7b upregulation in CD34+ cells failed to proof its effect on endothelial cells in vivo.

Conclusions—The present study demonstrates a significant upregulation of the angio-miRs miR-378 and let-7b in mobilized CD34+ progenitor cells of patients with STEMI. The increased proangiogenic activity of these cells in patients with STEMI and the observation that in particular miR-378 regulates the angiogenic capacity of CD34+ progenitor cells in vivo suggest that this unique miRNA expression pattern represents a novel endogenous repair mechanism activated in acute myocardial infarction. (***Arterioscler Thromb Vasc Biol*. 2017;37:341-349. DOI: 10.1161/ATVBAHA.116.308695.)**

Key Words: coronary artery disease ■ endothelial cells ■ microRNAs ■ myocardial infarction ■ real-time polymerase chain reaction

Experimental studies have demonstrated that administration or endogenous mobilization of bone marrow–derived progenitor cells limit cardiac injury and dysfunction after prolonged myocardial ischemia.[1,2] There is strong evidence, supporting the notion that vascular and proangiogenic effects of bone marrow–derived mononuclear or CD34+ progenitor cells play an important role in their capacity to ameliorate cardiac dysfunction after myocardial infarction (MI), based on a specific suicide gene approach or by blocking VEGF (vascular endothelial growth factor)

Received on: February 11, 2016; final version accepted on: November 28, 2016.

From the Department of Cardiology, University Heart Center (C.T., P.M., M.M., J.-R.G., M.J., C.M.M., T.F.L.), Department of Cardiovascular Surgery, Department of Surgical Research (M.Y.E., S.P.H.), University Hospital Zurich, Switzerland; Division of Nephrology and Hypertension, Department of Internal Medicine, Hannover Medical School, Germany (J.V.); Department of Cardiology, Campus Benjamin Franklin, Charité Universitätsmedizin Berlin, Germany (N.K., U.L.); Institute of Anatomy, University of Berne, Switzerland (B.S.-R., V.D.); Division of Cardiology, Kantonsspital Frauenfeld, Switzerland (M.M.); Division of Cardiology, Kerckhoff Klinik, Bad Nauheim, Germany (R.K.); Center for Molecular Cardiology, Schlieren Campus and Zurich Center of Integrative Human Physiology (ZIHP), University of Zurich, Switzerland (S.B., T.F.L.); First Department of Cardiology, Medical University of Gdansk, Poland (M.J.); Department of Cardiology, University of Geneva, Switzerland (F.M.); Department of Cardiology, University Hospital Bern, Switzerland (S.W.); Institute of Molecular and Translational Therapeutic Strategies (IMTTS), Hannover Medical School, Germany (T.T.); and National Heart and Lung Institute, Imperial College London, United Kingdom (T.T.).

*These authors contributed equally to this article.

The online-only Data Supplement is available with this article at http://atvb.ahajournals.org/lookup/suppl/doi:10.1161/ATVBAHA.116.308695/-/DC1.

Correspondence to Christian Templin, MD, Department of Cardiology, University Heart Center, University Hospital Zurich, Rämistr. 100, CH-8091 Zürich, Switzerland. E-mail Christian.Templin@usz.ch

Nonstandard Abbreviations and Acronyms	
ACS	acute coronary syndrome
miRNA/miR	microRNA
MI	myocardial infarction
PECAM-1	platelet endothelial cell adhesion molecule
sCAD	stable coronary artery disease
STEMI	ST-segment–elevation myocardial infarction
SuFu	suppressor of fused
VEGF	vascular endothelial growth factor

by specific antibodies.[3,4] Moreover, the administration of ex vivo expanded early outgrowth cells or CD34+ progenitor cells increases myocardial neovascularization in the border zone of the infarction and improves left ventricular ejection function after MI.[5,6] Fazel et al[7] have observed that mobilization of bone marrow–derived progenitor cells after MI was impaired in mice with a mutation of the c-kit receptor, and this was associated with a reduced cardiac neovascularization and more pronounced left ventricular dysfunction. These studies support the notion that endogenous bone marrow–derived progenitor cells are critical for the angiogenic switch in the infarct border zone, that is, the stimulation of cardiac neovascularization and preservation of cardiac function after MI. The proangiogenic capacity of progenitor cells in patients after acute coronary syndrome (ACS) is therefore likely important for their capacity to promote cardiac repair mechanisms. However, the exact molecular mechanisms contributing to the functional activation of progenitor cells mobilized in the context of an ACS remain to be defined.

Notably, microRNAs (miRNAs), small (≈22-nucleotide) noncoding RNAs have been identified as novel regulators of angiogenic processes, progenitor cell differentiation, and in particular cardiovascular cell fate decisions.[8–11] The crucial role of miRNAs in the cardiovascular system is further supported by the finding that depletion of the miRNA-processing enzyme Dicer leads to embryonic lethality because of defects in vessel formation and cardiac development.[12]

The present study was therefore designed to characterize the proangiogenic effects and differential expression of miRNAs regulating angiogenesis in CD34+ progenitor cells of patients with an acute ST-segment–elevation MI (STEMI) as compared with healthy subjects and patients with stable coronary artery disease (sCAD).

Materials and Methods

Materials and Methods are available in the online-only Data Supplement.

Results

Study Population

CD34+ progenitor cells were isolated prospectively from patients with acute and 5-day-old STEMI, patients with sCAD, and healthy subjects enrolled in the Swiss SPUM-ACS registry (www.spum-acs.ch). All 3 study groups were age and sex matched. The baseline characteristics of the study population are shown in the Table. Detailed information about the inclusion and exclusion criteria is described in the Materials and Methods in the online-only Data Supplement. The isolated CD34+ cells were CD45+ positive. A small number of circulating CD34+ cells, ≈1.5% were positive for CXCR4-receptor. Fluorescence-activated cell sorter results for CD34+ after the isolation are presented in Figure I in the online-only Data Supplement.

Proangiogenic Capacity of CD34+ Progenitor Cells From Patients With Acute STEMI Is Increased as Compared With Patients With sCAD and Healthy Subjects

The proangiogenic capacity of circulating CD34+ progenitor cells obtained from patients with STEMI at different time points after the event was examined and compared with circulating CD34+ progenitor cells of patients with sCAD and healthy subjects. We observed a significant increase in the proangiogenic capacity of CD34+ progenitor cells in patients with an acute STEMI as compared with patients with sCAD or healthy subjects (Figure 1A).

Differential miR-Expression in CD34+ Progenitor Cells From Patients With STEMI as Compared With sCAD and Healthy Subjects

MiRNAs have been identified as critical regulators of angiogenic pathways.[8–11] Thus, we performed a polymerase chain reaction–based miRNA array (including 372 miRs) to detect differentially regulated miRNAs in mobilized CD34+ progenitor cells of patients with STEMI as compared with those with sCAD or healthy subjects (Table I in the online-only Data Supplement). Thirty-six miRNAs were significantly deregulated in CD34+ progenitor cells of patients with STEMI as compared with healthy subjects (Figure II in the online-only Data Supplement). To investigate the proangiogenic function of CD34+ progenitor cells, we selected 2 known angiomiRs, let-7b and miR-378,[11,13,14] which were consistently upregulated in both, the real-time polymerase chain reaction screening array and the validation studies (Figure 2A and 2B), suggesting a potential role of these miRNAs in the regulation of proangiogenic effects of CD34+ progenitor cells. Other miRNAs of the validation studies, which were confirmed to be substantially upregulated in mobilized CD34+ progenitor cells after MI, were members from the miR-181-family, that is, miR-181a, miR-181b, and miR-181d (Figure 2C through 2E). Notably, miR-378, miR-181a, miR-181b, and miR-181d were significantly deregulated when compared with not only healthy subjects but also patients with sCAD (Figure 2A, 2C through 2E). No significant differences between the groups could be confirmed in the validation study for the other miRNAs (Figure III in the online-only Data Supplement).

Increased Proliferation of Pre–miR-378– and Pre–let-7b–Transfected CD34+ Cells in WST1 Proliferation Assay

It is known that the number of circulating CD34+ progenitor cells is increased in peripheral blood after MI.[15] Therefore, we performed in vitro proliferation experiments with WST1

Table. Characteristics of Study Population (n=27)

Study Groups	Healthy Subjects	sCAD	STEMI (Day 0)	*P* Value
Characteristics mean±SD				
Male, n (%)	7 (77.8)	7 (77.8)	7 (77.8)	1.000
Women, n (%)	2 (22.2)	2 (22.2)	2 (22.2)	1.000
Age, y	53.9±5.9	61.9±6.6	57.3±7.6	0.061
BP syst., mm Hg	120.0±9.3	142.1±15.6	134.6±25.7	0.048
BP diast., mm Hg	73.7±9.1	83.7±8.6	80.6±19.0	0.275
HR, bpm	62.9±8.0	76.6±10.7	79.8±15.1	0.012
CCS	0.0±0.0	2.0±0.9	4.0±0.0	<0.001
LVEF, %	60.9±4.0	54.3±15.4	54.3±17.3	0.510
BMI, kg/m^2	24.5±2.9	26.4±3.6	30.2±3.8	0.016
Laboratory values mean±SD				
Trop T, μg/L	<0.01	<0.01	2.2±2.6	<0.001
CK max, STEMI only			834.2±788.1	
CK-MB, U/L	11.4±3.9	15.1±7.9	74.9±77.9	0.014
NT-pro-BNP, ng/L	52.4±52.7	148.1±129.7	694.2±943.3	0.002
Creatinine, μmol/L	83.2±13.2	72.1±10.5	78.8±14.3	0.200
Hb, g/dL	14.0±0.9	13.9±1.5	13.3±2.1	0.603
WBC, 10^3/μL	5.5±0.9	7.1±2.1	10.1±3.4	0.001
Thrombocytes, 10^3/μL	286.8±45.6	268.2±100.4	248.6±73.7	0.578
Total cholesterol, mmol/L	5.6±0.9	4.9±1.4	4.5±1.5	0.202
LDL cholesterol, mmol/L	3.4±1.0	3.0±1.1	2.8±1.4	0.623
HDL cholesterol, mmol/L	1.7±0.5	1.3±0.3	1.1±0.3	0.006
Triglycerides, mmol/L	1.1±0.5	1.4±0.7	1.2±0.6	0.680
Medication, n (%)				
Statins	0.0 (0.0)	7.0 (77.8)	5.0 (55.6)	0.003
β-blockers	0.0 (0.0)	6.0 (66.7)	3.0 (33.3)	0.011
Diuretics	0.0 (0.0)	1.0 (11.1)	0.0 (0.0)	0.354
ACE-inhibitor	0.0 (0.0)	3.0 (33.3)	3.0 (33.3)	0.145
AT-I-antagonist	0.0 (0.0)	1.0 (11.1)	1.0 (11.1)	0.583
Calcium-channel blockers	0.0 (0.0)	1.0 (11.1)	0.0 (0.0)	0.354
Aspirin	0.0 (0.0)	8.0 (88.9)	4.0 (44.4)	<0.001
Clopidogrel	0.0 (0.0)	3.0 (33.3)	2.0 (22.2)	0.179

ACE indicates angiotensin-converting enzyme; AT-I, angiotensin II receptor type I; BMI, body mass index; BP, blood pressure; CCS, Canadian Cardiovascular Society; CK, creatine kinase; CK-MB, creatine kinase MB-isoenzyme; Hb, hemoglobin; HDL, high-density lipoprotein; HR, heart rate; LDL, low-density lipoprotein; NT-pro-BNP, N-terminal prohormone brain natriuretic peptide; sCAD, stable coronary artery disease; STEMI, ST-segment–elevation myocardial infarction; Trop T, troponinT; and WBC, white blood count.

reagent, which revealed significantly increased proliferation after 24 hours for transfected CD34+ cells with pre–miR-378 and pre–let-7b, compared with control transfected cells (Figure 3A; Figure IVA in the online-only Data Supplement). In contrast, anti-miR-transfection of miR-378 and let-7b (Figure 3B; Figure IVB in the online-only Data Supplement), as pre– and anti–miR-181a (Figure IVC and IVD in the online-only Data Supplement), showed no significant effect on CD34+ cell proliferation.

Increased Proangiogenic Capacity of miR-let-7b and miR-378 but not of miR-181a–Transfected CD34+ Cells in In Vitro Angiogenic Assays and Migration Assays

To determine whether miR-let-7b and miR-378 are regulating the angiogenic capacity of CD34+ progenitor cells, CD34+ cells obtained from healthy subjects were first transfected with the pre-miR or scramble-miR, respectively. The transfection efficiency is shown in Figure V in the online-only

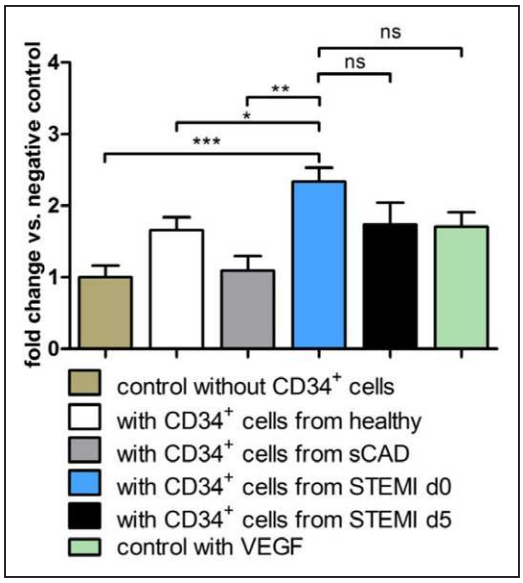


Figure 1. Proangiogenic effects of CD34+ cells from patients with ST-segment–elevation myocardial infarction (STEMI), stable coronary artery disease (sCAD), and healthy subjects. Human umbilical vein endothelial cell (1×10^4) were cocultured for 18 h in matrigel with starvation medium EBM-2 (endothelial basal medium type 2) containing 0.5% fetal calf serum and with CD34+ cells (5×10^3) from healthy subjects, patients with sCAD, with STEMI d0, with STEMI d5 or in starvation medium containing 50 ng/mL VEGF (vascular endothelial growth factor; as positive control) or without CD34+ cells (as negative control). Tube-like structures were quantified. *P<0.05, **P<0.01, ***P<0.001; data are mean±SEM.

Data Supplement. In vitro tube formation was significantly enhanced by coculture of human umbilical vein endothelial cells (HUVECs) with pre–miR-378–transfected and pre–miR-let-7b–transfected CD34+ cells as compared with scramble-transfected CD34+ cells. Moreover, downregulation of miR-378 and miR-let-7b in CD34+ cells using anti-miRs resulted in a reduced capacity of CD34+ progenitor cells to promote tube formation in comparison to scramble-miR–transfected CD34+ cells (Figure 4A; Figure VIA in the online-only Data Supplement). Despite, only HUVECs and not CD34+ progenitor cells form tube-like structures (Figure VII in the online-only Data Supplement).

In addition, using the endothelial scratch migration assay, we observed that CD34+ cells transfected with pre–miR-378 or pre–miR-let-7b had an increased capacity to stimulate endothelial migration as measured by the endothelial migration index i (Figure 4B; Figure VIB in the online-only Data Supplement).

To date, the effects of miR-181a on angiogenesis have not been reported yet. Therefore, we performed angiogenesis tube formation assays in HUVECs transfected with pre–miR-181a and anti–miR-181a to determine its impact on angiogenesis and endothelial cells. Pre–miR-181a–transfected HUVECs showed a significantly decreased tube formation capacity, whereas anti–miR-181a transfection led to an increased tube formation activity (Figure VIII in the online-only Data Supplement).

In summary, the present findings are compatible with the notion that both miRNAs—let-7b and miR-378—regulate and augment the proangiogenic capacity of CD34+ cells and their stimulatory effects on vascular endothelial cells in vitro. Because of the marked antiangiogenic effects of miR-181a in endothelial cells, we did not perform further angiogenic experiments for this miRNA in CD34+ cells.

Paracrine Proangiogenic Impact of CD34+ Cells

For a long time, the proangiogenic capacity of CD34+ cells was associated with paracrine cytokine release.[16] In our study, we performed an ELISA-based angiogenic cytokine Bioplex-assay with supernatant, collected from transfected CD34+ cells. For PECAM-1 (platelet endothelial cell adhesion molecule), VEGF, leptin, and follistatin, we observed a nonsignificant increase after CD34+ cell transfection with pre–miR-378 or pre–let-7b (Figure IXa and IXb in the online-only Data Supplement).

Microparticle-mediated transfer of miRNAs between different cell types is a possible mechanism for the paracrine effects.[17] Thus, we performed a coculture assay containing transfected CD34+ cells and HUVECs, measuring the concentration of selected miRNAs in the supernatant and HUVECs. Our results proofed equal miRNA-secretion of pre-miR–transfected CD34+ cells (Figure 5A through 5C), whereas significant miRNA uptake in HUVECs could only be demonstrated for miR-378 and miR-181a, but not for let-7b (Figure 5D and 5E).

Augmented Angiogenic Capacity of Pre–miR-378–Transfected Cells in In Vivo Angiogenic Experiments: Matrigel Plug Assay and Chorioallantoic Membrane Assay

We focused in our in vivo experiments on the proangiogenic function of miR-378. To characterize the role of miR-378 in CD34+ cells in vivo, we performed Matrigel plug assay with pre– and anti–miR-378–transfected CD34+ cells. Strikingly, pre–miR-378–transfected CD34+ cells significantly increased the amount of blood vessel sprouting in the matrigel plug, compared with scramble, whereas anti–miR-378 transfection resulted in significantly decreased blood vessel sprouting (Figure 6A). Notably, let-7b–transfected CD34+ cells did not impact in vivo blood vessel sprouting (Figure X in the online-only Data Supplement).

Moreover, similar results for pre–miR-378–transfected CD34+ progenitor cells could be verified by chicken embryo in vivo angiogenesis assay. Increased in vivo blood vessel sprouting was observed after incubation of chorioallantoic membrane with pre–miR-378–transfected CD34+ cells as compared with scrambled-miR–transfected CD34+ cells (Figure 6B).

Discussion

We here report that in mobilized CD34+ cells obtained from patients with STEMI miRNAs are differentially regulated. In our polymerase chain reaction–based miRNA-profiling analysis and subsequent validation studies with real-time polymerase chain reaction, we observed a significant upregulation of 2 known angio-miRs, miR-378, and let-7b, in CD34+ progenitor cells obtained from patients with STEMI at the time of presentation, compared with healthy subjects and in

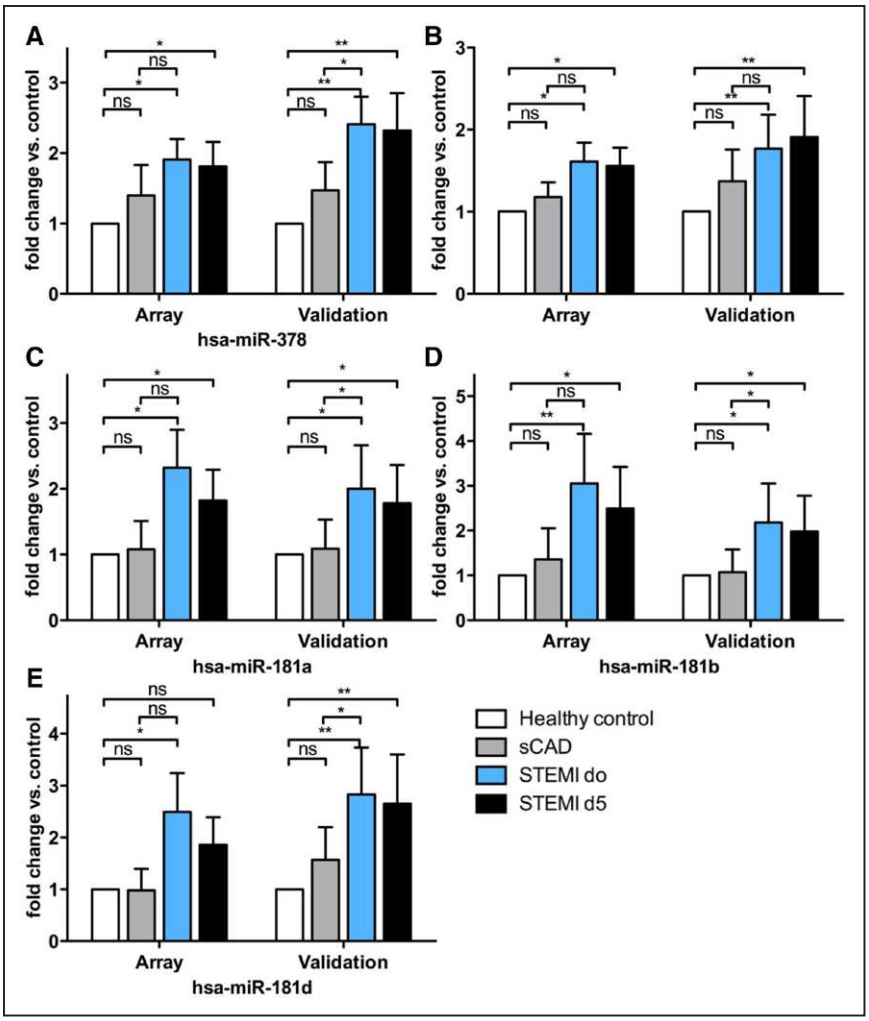


Figure 2. Results of the miRNA profiling and validation with real-time polymerase chain reaction in CD34+ progenitor cells. MicroRNA expression levels for the miRNAs: **A**, miR-378, (**B**) let-7b, (**C**) miR-181a, (**D**) miR-181b, (**E**), miR-181d in CD34+ progenitor cells isolated from healthy subjects, patients with stable coronary artery disease (sCAD), with ST-segment–elevation myocardial infarction (STEMI) d0, with STEMI d5. **P*<0.05, ***P*<0.01, data are mean±SEM.

addition only for miR-378 compared with sCAD. Especially, we identified miR-378 as critical regulator of the proangiogenic capacity of CD34+ progenitor cells and its stimulatory effects on endothelial cells in vitro and in vivo, whereas let-7b upregulation in CD34+ cells failed to exhibit relevant effects on endothelial cells in vivo.

The CD34+CD45+ progenitor cells population is also defined as early endothelial progenitor cells or as angiogenic cells. Early endothelial progenitor cells enhance neovascularization indirectly via paracrine effects.[18]

Bone marrow–derived CD34+ progenitor cells are mobilized in patients with an acute MI or in response to myocardial ischemia.[15,19,20] In this context, the investigated angio–miR-378 proofed to markedly increase CD34+ cell proliferation, as measured by the WST1 reaction. Mobilization of CD34+ progenitor cells from the bone marrow into peripheral blood is associated with acute and chronic preservation of left ventricular function in patients with MI.[19,21] Therefore, increased CD34+ cell count in response to miR-378–mediated proliferation supports the angiogenic effect of CD34+ cells in the context of an acute MI.

Indeed, several in vitro and in vivo studies have reported a proangiogenic effect of CD34+ cells.[4,6] In particular, the effects of administration of bone marrow–derived mononuclear cells or CD34+ progenitor cells on recovery of left ventricular function after experimental MI have recently been related to their proangiogenic effects.[3]

Consistently, miRNAs, particularly angio-miRs, have been shown to regulate the proliferation, migration, and angiogenic capacity of endothelial cells.[11,22] Xing et al[23] demonstrated an increased proliferation and in vitro angiogenesis in mesenchymal cells under hypoxic conditions after miR-378 mimic

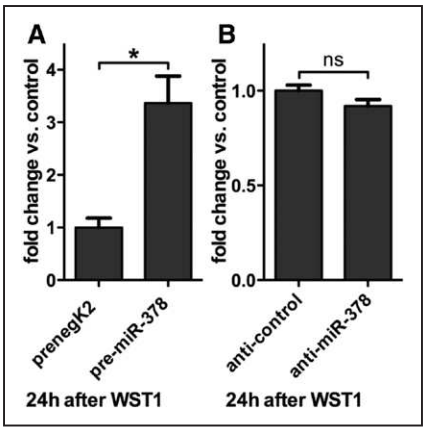


Figure 3. Proliferation assay. Proliferation of CD34+ progenitor cells (1×10[4]) transfected with (**A**) pre–miR-378 and (**B**) anti–miR-378, quantified with WST1 proliferation reagent. **P*<0.05, data are mean±SEM.

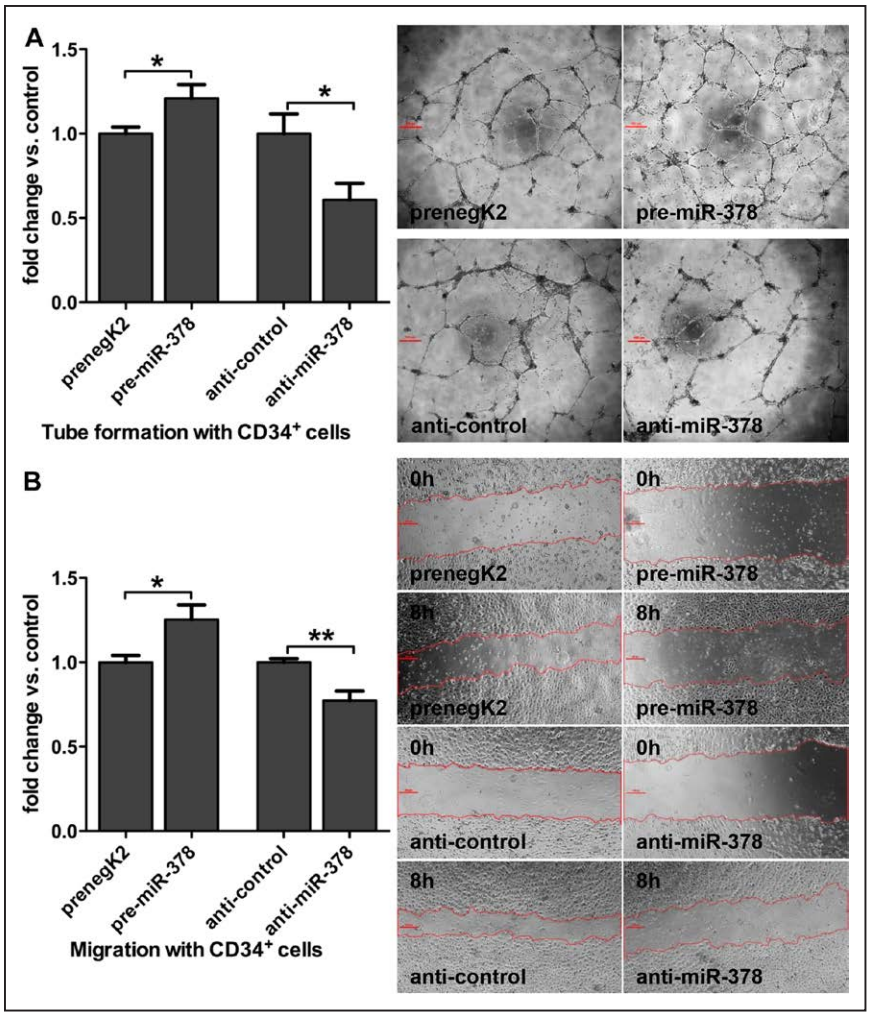


Figure 4. In vitro assays with scramble-transfected CD34⁺ cells compared with pre–miR-378/anti–miR-378–transfected CD34⁺ cells. **A**, Tube formation angiogenesis assay with human umbilical vein endothelial cells (HUVECs; 1×10^4) cocultered for 18 h with CD34⁺-transfected cells (5×10^3). Tube-like structures were quantified. **B**, Endothelial scratch migration assay. After scratching, HUVECs were cultured in starvation medium with scramble/pre–miR-378–/anti–miR-378–transfected CD34⁺ cells (4×10^4). The stimulatory effect of transfected CD34⁺ cells on endothelial cell migration capacity was measured using the migration index (8 h after scratch). *P<0.05, **P<0.01, data are mean±SEM.

transfection. The present study further demonstrates a marked proangiogenic activation of CD34⁺ cells in patients with an acute STEMI and identifies the angio-miRs miR-378 and let-7b, which are profoundly upregulated in these cells. To further investigate the functional role of these miRs with regards to proangiogenic capacity, CD34⁺ cells obtained from healthy subjects were transfected with pre–miR-378 and pre–miR-let-7b or scrambled miRs. Elevated expression of miR-378 and let-7b increased the proangiogenic capacity of CD34⁺ cells in vitro by inducing tube formation. Moreover, transfection of CD34⁺ progenitor cells with both miRNAs (but not with scrambled miR) increased their stimulatory effect on endothelial cells, that is, endothelial cell migration. Vice versa, downregulation of these angio-miRs using anti-miRs reduced the proangiogenic effects and migration effects of CD34⁺ progenitor cells.

The regulation of angio-miR expression in CD34⁺ cells and the mechanism of interaction between endothelial cells and stem/progenitor cells are not fully understood. Recent evidence suggests that CD34⁺ cells facilitate angiogenesis largely via paracrine effects.[16]

Hence, we performed the ELISA-based Bioplex Angiogenic Cytokine assay in supernatant of transfected CD34⁺ cells to investigate, whether the proangiogenic effects of angio-miRs in CD34⁺ cells are based on their paracrine secretion of proangiogenic cytokines. Our results revealed no significant changes, only a trend for increased release of follistatin, PECAM-1, leptin, and VEGF in response to miR-378 or let-7b overexpression.

Extracellular miRNAs were identified as a new signaling pathway, which could be selectively exported for cell-to-cell communication.[24] These extracellular miRs must be protected against degradation by exonucleases. Therefore, they are included into microparticles such as exosomes, microvesicles, or apoptotic bodies or they are attached to protein complexes like argonaute 2 or lipoprotein-complexes.[17] Sahoo et al[25] have reported that exosomes, secreted by CD34⁺ cells, improve angiogenesis in vivo and in vitro and that several angio-miRs are enriched in their exosomes. Accordingly, we performed coculturing experiments of transfected CD34⁺ cells and HUVECs, which revealed that CD34⁺ cell-released angio-miR-378, but not let-7b, is taken up by HUVECs. Thus, transport of angio-miR-378 by microparticles released from CD34⁺ cells represents a novel, paracrine mechanism, contributing to their angiogenic effects on endothelial cells by direct endocytosis of the miR-378. These angio-miR–containing microparticles therefore likely represent an important mode of intercellular communication and exchange.

MiR-378 is enriched in CD34⁺ hematopoietic progenitor cells[26] and has been suggested to promote angiogenesis by

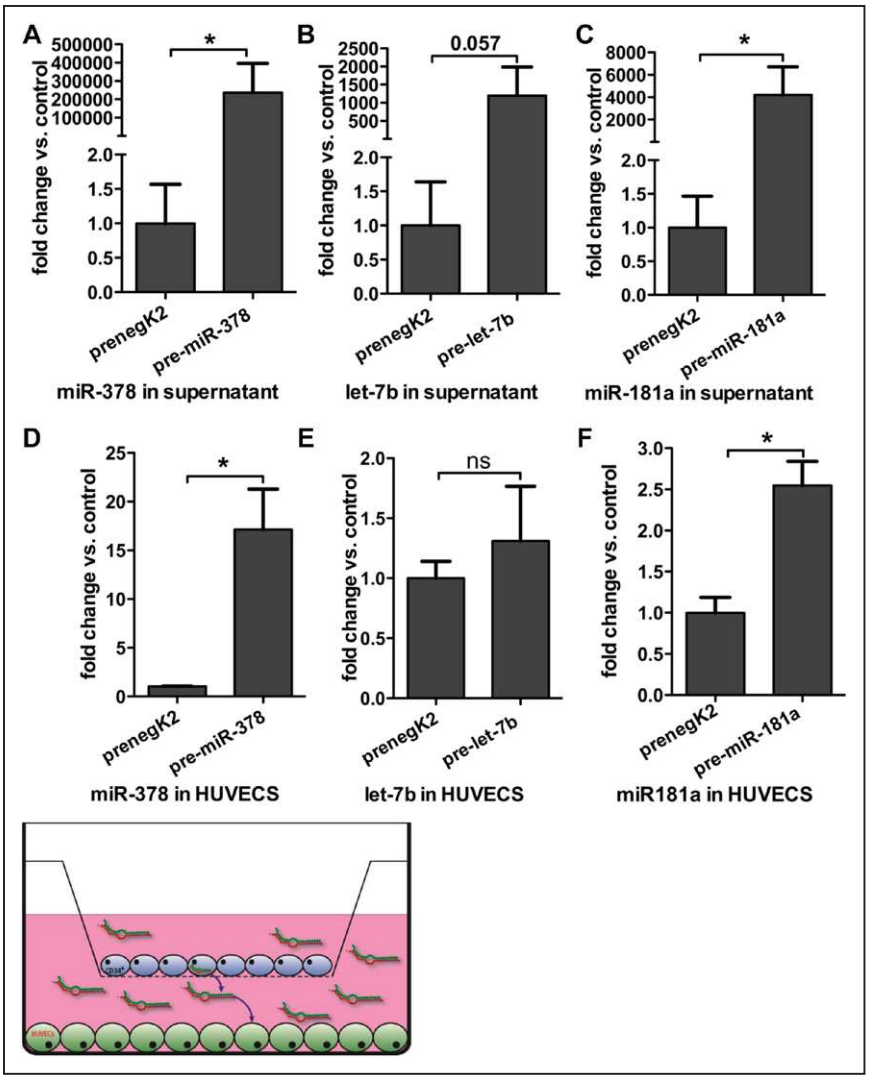


Figure 5. Coculture assays. MiRNA expression of (**A** and **D**) miR-378, (**B** and **E**) let-7b, and (**C** and **F**) miR-181a in supernatant and in human umbilical vein endothelial cell (HUVECs) after coculture with pre–miR-378–/miR-let-7b–/miR-181–transfected CD34+ cells separated by 4-µm transwell inserts. *P<0.05, data are mean±SEM.

targeting Suppressor of fused (SuFu) and Fus-1 expression and regulates cell survival.[14] Moreover, nude mice injected with miR-378–transfected cancer cells formed substantially larger blood vessels as compared with GFP (green fluorescent protein)-transfected cells.[14] Furthermore, miR-378 has been reported to promote VEGF expression by competing with miR-125a for the same seed region in the VEGF 3′-untranslated region.[14] Sufu and Fus-1 serve as targets for miR-378 repression. Sufu functions as a negative regulator of Sonic hedgehog (Shh) signaling pathway. Shh promotes vessel formation by inducing expression of angiogenic cytokines, including VEGF and angiopoietin-1 (Ang-1) and -2 (Ang-2).[27] Therefore, miRNA-378 may promote angiogenesis by targeting Sufu and Fus-1, and hence indirectly upregulates angiogenic factors.

Based on the results of the paracrine mechanism-demonstrating experiments, we decided to focus our in vivo studies on miR-378–transfected CD34+ cells, offering the highest impact on paracrine angiogenic cytokine release and on angiomiR-endothelial cell uptake.

Taken together, we transferred our recent findings from in vitro–based assays to in vivo angiogenesis models, such as in vivo matrigel plug assay and chorioallantoic membrane assay. Therefore, miR-378–transfected CD34+ cells significantly increased blood vessel infiltration and blood vessel sprouting, in support of our hypothesis. A limitation of the current miR-mimic experiments is the fact that substantially increased miR-378 levels may overestimate the effects of more moderate changes of this miR in patients with MI.

Urbich et al[28] indicated that CD34+ or CD 133+ EPCs from patients with sCAD show reduced endothelial regenerative function and impaired neovascularization. In line with this, our findings suggest an impaired angiogenic capacity for CD34+ cells in patients with sCAD. In this regard, mobilized and isolated CD34+ cells could be transfected by pre–miR-378 to stimulate its proangiogenic capacity. Using this mechanisms, the augmented proangiogenic capacity of CD34+ cells after STEMI could be transferred to CD34+ cells in patients with sCAD.

Moreover, our findings further indicate that the miR181-family is strongly upregulated in CD34+ progenitor cells of patients with acute STEMI. More recently, it has been suggested that increasing miR-181a levels stimulates deprogramming of lymphatic endothelial cells toward a blood vascular phenotype by targeting Prox1.[29] After MI, the

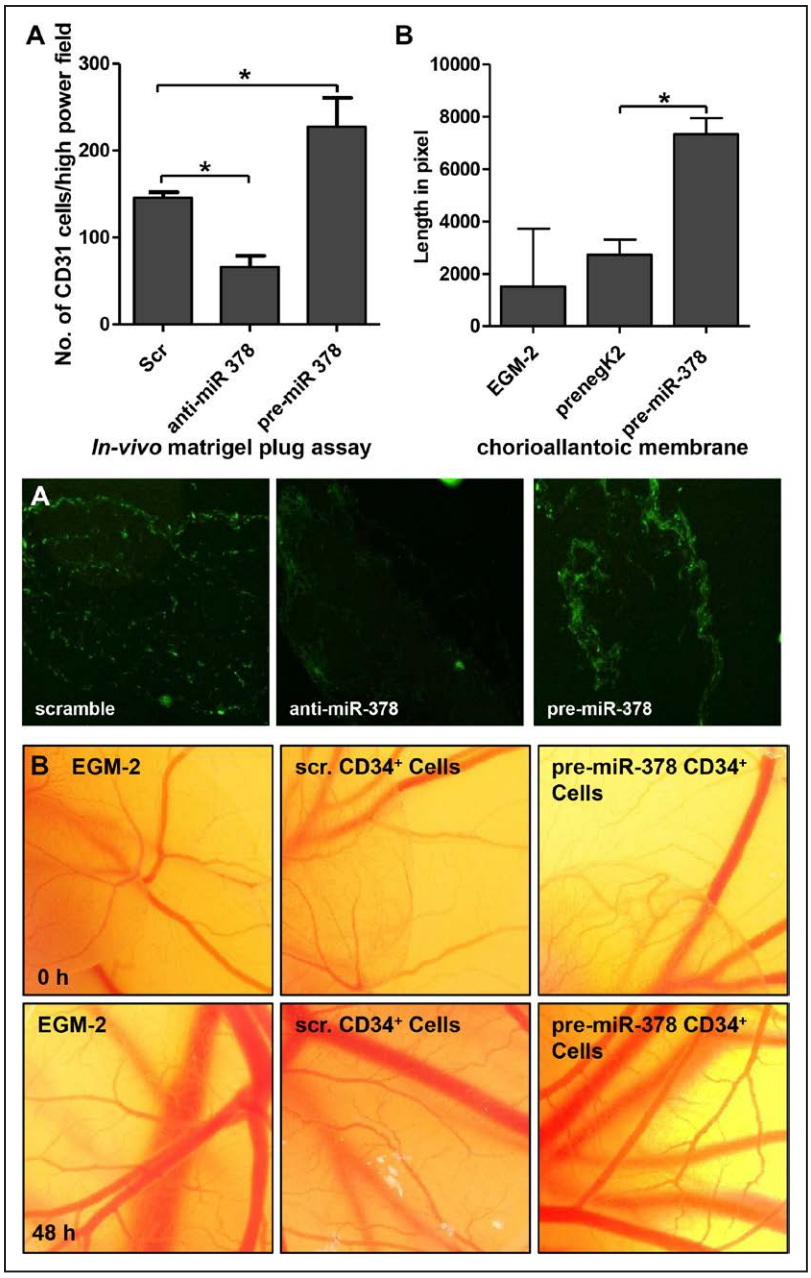


Figure 6. In-vivo assays. **A**, In vivo matrigel plug assay. Blood vessel infiltration in Matrigel plugs containing (1×10^5) scramble/pre–miR-378–/anti–miR-378–transfected CD34⁺ cells was quantified by analysis of CD31-staining 5 d after injection. **B**, Chorioallantoic membrane (CAM) angiogenesis assay in vivo. Difference of the vessel length 48 h after incubation with EGM-2 (endothelial growth medium type 2) medium, scramble-transfected CD34⁺ cells (scr. CD34⁺ cells) or with pre–miR-378–transfected CD34⁺ cells (pre–miR-378 CD34⁺ cells). *P<0.05, data are mean±SEM.

miR-181 family is enriched in CD34⁺ cells and our coculture experiments demonstrated uptake of secreted miR-181a in endothelial cells. The role of upregulation of the miR-181 family members for the differentiation of endothelial cells and blood vessel formation needs to be further elucidated in future studies.

In conclusion, we have identified miR-378 and partly let-7b as differentially regulated miRNAs in circulating CD34⁺ progenitor cells from patients with STEMI, which critically modulate the proangiogenic and paracrine capacity of these cells. Furthermore, we investigated a link between miR-181 family and MI. These findings reveal a novel mechanism contributing to the proangiogenic activation of CD34⁺ progenitor cells in patients with an acute STEMI. Moreover, a novel use of pre–miR-378–transfected CD34⁺ cells in stem cell therapy for patients with sCAD seems possible.

Acknowledgments

We thank Anika Lewandowski for their excellent technical assistance in this study.

Sources of Funding

The study was supported by grants of the Swiss National Research Foundation Sonderprogramm Universitäre Medizin [Nr. 33CM30-124112/1], the Uniscientia Foundation, the IFB-Tx (BMBF 01EO0802), by the Zurich Center for Integrative Human Physiology, the DFG TH 903/7-2, AstraZeneca, Zug, Switzerland, Eli Lilly, Indianapolis, Medtronic, Tollachenaz, Switzerland and the Zurich Heart House—Foundation for Cardiovascular Research. N. Kraenkel was supported by the fellowships PZ00P3_126621 and PZ00P3_142300 of the Swiss National Science Foundation.

Disclosures

None.

References

1. Templin C, Kränkel N, Lüscher TF, Landmesser U. Stem cells in cardiovascular regeneration: from preservation of endogenous repair to future cardiovascular therapies. *Curr Pharm Des*. 2011;17:3280–3294.
2. Templin C, Lüscher TF, Landmesser U. Cell-based cardiovascular repair and regeneration in acute myocardial infarction and chronic ischemic cardiomyopathy-current status and future developments. *Int J Dev Biol*. 2011;55:407–417. doi: 10.1387/ijdb.103219ct.
3. Yoon CH, Koyanagi M, Iekushi K, Seeger F, Urbich C, Zeiher AM, Dimmeler S. Mechanism of improved cardiac function after bone marrow mononuclear cell therapy: role of cardiovascular lineage commitment. *Circulation*. 2010;121:2001–2011. doi: 10.1161/CIRCULATIONAHA.109.909291.
4. Wang J, Zhang S, Rabinovich B, Bidaut L, Soghomonyan S, Alauddin MM, Bankson JA, Shpall E, Willerson JT, Gelovani JG, Yeh ET. Human CD34+ cells in experimental myocardial infarction: long-term survival, sustained functional improvement, and mechanism of action. *Circ Res*. 2010;106:1904–1911. doi: 10.1161/CIRCRESAHA.110.221762.
5. Asahara T, Masuda H, Takahashi T, Kalka C, Pastore C, Silver M, Kearne M, Magner M, Isner JM. Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathological neovascularization. *Circ Res*. 1999;85:221–228.
6. Kawamoto A, Iwasaki H, Kusano K, Murayama T, Oyamada A, Silver M, Hulbert C, Gavin M, Hanley A, Ma H, Kearney M, Zak V, Asahara T, Losordo DW. CD34-positive cells exhibit increased potency and safety for therapeutic neovascularization after myocardial infarction compared with total mononuclear cells. *Circulation*. 2006;114:2163–2169. doi: 10.1161/CIRCULATIONAHA.106.644518.
7. Fazel S, Cimini M, Chen L, Li S, Angoulvant D, Fedak P, Verma S, Weisel RD, Keating A, Li RK. Cardioprotective c-kit+ cells are from the bone marrow and regulate the myocardial balance of angiogenic cytokines. *J Clin Invest*. 2006;116:1865–1877. doi: 10.1172/JCI27019.
8. van Rooij E, Olson EN. MicroRNAs: powerful new regulators of heart disease and provocative therapeutic targets. *J Clin Invest*. 2007;117:2369–2376. doi: 10.1172/JCI33099.
9. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell*. 2004;116:281–297.
10. Georgantas RW 3rd, Hildreth R, Morisot S, Alder J, Liu CG, Heimfeld S, Calin GA, Croce CM, Civin CI. CD34+ hematopoietic stem-progenitor cell microRNA expression and function: a circuit diagram of differentiation control. *Proc Natl Acad Sci U S A*. 2007;104:2750–2755. doi: 10.1073/pnas.0610983104.
11. Heinrich EM, Dimmeler S. MicroRNAs and stem cells: control of pluripotency, reprogramming, and lineage commitment. *Circ Res*. 2012;110:1014–1022. doi: 10.1161/CIRCRESAHA.111.243394.
12. Kuehbacher A, Urbich C, Zeiher AM, Dimmeler S. Role of Dicer and Drosha for endothelial microRNA expression and angiogenesis. *Circ Res*. 2007;101:59–68. doi: 10.1161/CIRCRESAHA.107.153916.
13. Wang S, Olson EN. AngiomiRs–key regulators of angiogenesis. *Curr Opin Genet Dev*. 2009;19:205–211. doi: 10.1016/j.gde.2009.04.002.
14. Lee DY, Deng Z, Wang CH, Yang BB. MicroRNA-378 promotes cell survival, tumor growth, and angiogenesis by targeting SuFu and Fus-1 expression. *Proc Natl Acad Sci U S A*. 2007;104:20350–20355. doi: 10.1073/pnas.0706901104.
15. Wojakowski W, Tendera M, Michałowska A, Majka M, Kucia M, Maślankiewicz K, Wyderka R, Ochała A, Ratajczak MZ. Mobilization of CD34/CXCR4+, CD34/CD117+, c-met+ stem cells, and mononuclear cells expressing early cardiac, muscle, and endothelial markers into peripheral blood in patients with acute myocardial infarction. *Circulation*. 2004;110:3213–3220. doi: 10.1161/01.CIR.0000147609.39780.02.
16. Scheubel RJ, Holtz J, Friedrich I, Borgermann J, Kahrstedt S, Navarrete Santos A, Silber RE, Simm A. Paracrine effects of CD34 progenitor cells on angiogenic endothelial sprouting. *Int J Cardiol*. 2010;139:134–141. doi: 10.1016/j.ijcard.2008.10.009.
17. Kinet V, Halkein J, Dirkx E, Windt LJ. Cardiovascular extracellular microRNAs: emerging diagnostic markers and mechanisms of cell-to-cell RNA communication. *Front Genet*. 2013;4:214. doi: 10.3389/fgene.2013.00214.
18. Timmermans F, Van Hauwermeiren F, De Smedt M, Raedt R, Plasschaert F, De Buyzere ML, Gillebert TC, Plum J, Vandekerckhove B. Endothelial outgrowth cells are not derived from CD133+ cells or CD45+ hematopoietic precursors. *Arterioscler Thromb Vasc Biol*. 2007;27:1572–1579. doi: 10.1161/ATVBAHA.107.144972.
19. Leone AM, Rutella S, Bonanno G, Abbate A, Rebuzzi AG, Giovannini S, Lombardi M, Galiuto L, Liuzzo G, Andreotti F, Lanza GA, Contemi AM, Leone G, Crea F. Mobilization of bone marrow-derived stem cells after myocardial infarction and left ventricular function. *Eur Heart J*. 2005;26:1196–1204. doi: 10.1093/eurheartj/ehi164.
20. Porto I, Leone AM, De Maria GL, Hamilton Craig C, Tritarelli A, Camaioni C, Natale L, Niccoli G, Biasucci LM, Crea F. Are endothelial progenitor cells mobilized by myocardial ischemia or myocardial necrosis? A cardiac magnetic resonance study. *Atherosclerosis*. 2011;216:355–358. doi: 10.1016/j.atherosclerosis.2011.02.014.
21. Wojakowski W, Tendera M, Zebzda A, Michalowska A, Majka M, Kucia M, Maslankiewicz K, Wyderka R, Król M, Ochala A, Kozakiewicz K, Ratajczak MZ. Mobilization of CD34(+), CD117(+), CXCR4(+), c-met(+) stem cells is correlated with left ventricular ejection fraction and plasma NT-proBNP levels in patients with acute myocardial infarction. *Eur Heart J*. 2006;27:283–289. doi: 10.1093/eurheartj/ehi628.
22. Suárez Y, Sessa WC. MicroRNAs as novel regulators of angiogenesis. *Circ Res*. 2009;104:442–454. doi: 10.1161/CIRCRESAHA.108.191270.
23. Xing Y, Hou J, Guo T, Zheng S, Zhou C, Huang H, Chen Y, Sun K, Zhong T, Wang J, Li H, Wang T. microRNA-378 promotes mesenchymal stem cell survival and vascularization under hypoxic-ischemic conditions *in vitro*. *Stem Cell Res Ther*. 2014;5:130. doi: 10.1186/scrt520.
24. Boon RA, Vickers KC. Intercellular transport of microRNAs. *Arterioscler Thromb Vasc Biol*. 2013;33:186–192. doi: 10.1161/ATVBAHA.112.300139.
25. Sahoo S, Klychko E, Thorne T, Misener S, Schultz KM, Millay M, Ito A, Liu T, Kamide C, Agrawal H, Perlman H, Qin G, Kishore R, Losordo DW. Exosomes from human CD34(+) stem cells mediate their proangiogenic paracrine activity. *Circ Res*. 2011;109:724–728. doi: 10.1161/CIRCRESAHA.111.253286.
26. Bruchova H, Yoon D, Agarwal AM, Mendell J, Prchal JT. Regulated expression of microRNAs in normal and polycythemia vera erythropoiesis. *Exp Hematol*. 2007;35:1657–1667. doi: 10.1016/j.exphem.2007.08.021.
27. Pola R, Ling LE, Silver M, Corbley MJ, Kearney M, Blake Pepinsky R, Shapiro R, Taylor FR, Baker DP, Asahara T, Isner JM. The morphogen Sonic hedgehog is an indirect angiogenic agent upregulating two families of angiogenic growth factors. *Nat Med*. 2001;7:706–711. doi: 10.1038/89083.
28. Urbich C, Kuehbacher A, Dimmeler S. Role of microRNAs in vascular diseases, inflammation, and angiogenesis. *Cardiovasc Res*. 2008;79:581–588. doi: 10.1093/cvr/cvn156.
29. Kazenwadel J, Michael MZ, Harvey NL. Prox1 expression is negatively regulated by miR-181 in endothelial cells. *Blood*. 2010;116:2395–2401. doi: 10.1182/blood-2009-12-256297.

Highlights

- Currently, CD34+ progenitor cells are used to increase revascularization after myocardial infarction.
- We investigated miRNAs involved in this process in isolated cells from patients with myocardial infarction and determined the proangiogenic miRNA-378, which proofed to be essential for the already known proangiogenic role of CD34+ progenitor cells.
- We successfully validated the proangiogenic impact of this miRNA on CD34+ progenitor cells derived from healthy subjects in vitro and in vivo.
- Our findings may lead to new therapeutic options in patients after myocardial infarction.

Arteriosclerosis, Thrombosis, and Vascular Biology

JOURNAL OF THE AMERICAN HEART ASSOCIATION

Increased Proangiogenic Activity of Mobilized CD34$^+$ Progenitor Cells of Patients With Acute ST-Segment–Elevation Myocardial Infarction: Role of Differential MicroRNA-378 Expression

Christian Templin, Julia Volkmann, Maximilian Y. Emmert, Pavani Mocharla, Maja Müller, Nicolle Kraenkel, Jelena-R. Ghadri, Martin Meyer, Beata Styp-Rekowska, Sylvie Briand, Roland Klingenberg, Milosz Jaguszewski, Christian M. Matter, Valentin Djonov, Francois Mach, Stephan Windecker, Simon P. Hoerstrup, Thomas Thum, Thomas F. Lüscher and Ulf Landmesser

Arterioscler Thromb Vasc Biol. 2017;37:341-349; originally published online December 29, 2016;
doi: 10.1161/ATVBAHA.116.308695
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 1079-5642. Online ISSN: 1524-4636

Material and Methods

Study population

$CD34^+$ progenitor cells were isolated prospectively from patients with acute STEMI diagnosed according to the ESC/AHA redefined guidelines(1), patients with stable CAD and healthy subjects. Patients with STEMI had a duration of chest pain of below 24 hours from the onset of symptoms till the presentation in the catheterization laboratory. $CD34^+$ progenitor cells were isolated at baseline (day 0) and at day 5. Patients with stable CAD had angiographically documented stenosis above 50 % in at least one coronary vessel. Patients were excluded from the present study when one of the following criteria were present: Known history of leucopenia, thrombocytopenia, severe hepatic or renal dysfunction, heart failure, evidence of inflammatory or malignant disease as well as myocardial infarction within the last 6 months in the sCAD group. Healthy subjects had no known cardiovascular disease, normal cardiac function validated by echocardiography, no structural heart disease, did not take any medication nor were smokers. The study protocol was carried out according to the principles of the Declaration of Helsinki and was approved by the local ethic committee. Written informed consent was obtained from all patients and healthy subjects before enrollment.

Isolation of $CD34^+$ progenitor cells and *in-vitro* culture

$CD34^+$ cells were isolated from 60 ml of anticoagulated peripheral blood (BD Vacutainer, K2EDTA 18.0 mg) or from Buffy Coats using the CD34-MicroBead-Kit (Miltenyi Biotec) according to the manufacturer's protocol. In brief, peripheral blood mononuclear cells (PBMC) were isolated by density gradient centrifugation for 30min at 400g with LSM 1077 (PAA Laboratories) and labeled with anti-CD34 magnetic beads using two successive separations over LS MACS columns. For the functional evaluation $CD34^+$ cells isolated from Buffy Coats were cultured in serum-free HPGM™ (Hematopoietic Progenitor Growth Medium; Lonza) supplemented with 50 ng/ml Flt-3L (Peprotech), 25ng/ml stem cell factor (SCF, Peprotech) and 50ng/ml TPO (Peprotech) as described previously with some minor modifications.(2) The amount of the $CD34^+$ cells after the isolation from patients was between 10^5 and 1,5 X 10^5 and from Buffy Coats between 5 X 10^5 and 8 X 10^5.

RNA Isolation, microRNA Array and Real-Time PCR Analysis

Total RNA was isolated from $CD34^+$ cells from patients with sCAD, STEMI events or healthy subjects using QIAzol Reagent (Qiagen) and miRNeasy Mini Kit (Qiagen) according to the manufacturer's instructions. Differential miR-expression in $CD34^+$ cells was analysed using PCR-based miRNA profiling on Human panel I plates from Exiqon. Differential expression of individual miRs between the patient groups (STEMI at day 0 and day 5, sCAD and healthy subjects) was validated using Real-Time PCR analysis according to miRCURY LNA™ Universal RT microRNA PCR protocol (Exiqon). cDNA was synthesized from 5ng of total RNA with Universal cDNA Synthesis Kit (Exiqon), according to the manufacturer's instruction.

Real-Time PCR was performed using the SYBR Green master mix (Exiqon) and for each microRNA specific LNA PCR primer set (Exiqon) with a MX3000P PCR cycler. The qPCR data were normalized to the miR-103-values and relative miRNA expression was calculated using the $2^{-\Delta\Delta Ct}$ method.

Transfection of CD34+ progenitor cells with miR-mimic and anti-miR

$CD34^+$ progenitor cells isolated from Buffy Coats (leukocytes and thrombocytes from blood donations) were resuspended in antibiotic-free growth medium and transfected using 2µL Lipofectamine 2000 (Invitrogen) with 50-100nM miR-mimic (pre-miR) or anti-miR of miR-378, let-7b and miR-181a or with scramble-miR (prenegK2 for miR-mimic control and anti-control for anti-miR control, Ambion), using Lipofectamine 2000 (Invitrogen) according to manufacturer's protocol. Four hours after incubation, transfection medium was removed and transfected $CD34^+$ cells were seeded in 12-well suspension cell culture plates with fresh growth medium. Transfected $CD34^+$ cells were used for the experiments after 48 hours.

Transfection efficiency
$CD34^+$ progenitor cells were transfected in antibiotic-free growth medium using 2µL Lipofectamine 2000 (Invitrogen) with 100nM Cy3 labeled pre-miR positive control and anti-miR negative control (Ambion) according to manufacturer's protocol. 4 hours after transfection $CD34^+$ cells were washed stained with DAPI (1:1000) for additional 2 hours. Afterwards, transfection efficacy was quantified by fluorescence imaging and FACS-measurement for total Cy3 positive cells.

Proliferation assay
WST1-proliferation assay (Roche) was measured in $CD34^+$ progenitor cells isolated from Buffy Coats according to manufacturer protocol. In detail, $1*10^4$ transfected $CD34^+$ cells were cultured for 24 hours in culture medium, containing 10% WST1 proliferation reagent. The proliferation measurement was performed by reading absorbance at 450nm.

Matrigel angiogenesis assay *in-vitro* (tube formation)
Human umbilical vein endothelial cells (HUVECs, Lonza) were cultured in endothelial cell growth medium (EGM-2: endothelial cell basal medium (EBM-2) with SingleQuots®, Lonza) with 10% FCS before being plated in 96-well plates ($1x10^4$ cells per well) previously coated with 25µl of Matrigel (growth factor-reduced; BD Biosciences). $CD34^+$ cells ($5x10^3$ cells per well) from the study subjects or transfected $CD34^+$ cells from Buffy Coats ($5x10^3$ cells per well) in 100µl of EBM-2 medium with 1% FCS were added for co-culture. After 18h of culture, tube-like structures were visualized and quantified per well using an inverted cell culture microscope and a digital camera (Nikon Ti-90) at 4x magnification as described in detail previously.(3)

Scratch migration assay
HUVECs ($5x10^4$ per well) were seeded into 12-well plates and cultured in EGM-2 Medium with 10% FCS until cells form 90%–100% confluent monolayers. The scratch was performed with sterile P10 pipette tips. HUVECs were washed with PBS to remove cellular debris and floating cells, and cultured with transfected $CD34^+$ cells from Buffy Coats ($4x10^4$ cells per well) in 1 ml EBM-2 Medium containing 1% FCS. The cell migration capacity was measured as migration index I for quantification of total migration capacity, representing not only distance but total area for migration. This was described earlier by Menon et al.(4) Therefore, the scratched area was photographed under an inverted microscope (Nikon Ti-90) at the time points 0 and 8 hours.

Cytokine assay
ELISA-based Bioplex-Cytokine assay (Bio-Rad) was performed per manufacturer instructions. Therefore, $6*10^5$ transfected $CD34^+$ cells from Buffy Coats cells were cultured for 48 h and supernatant was collected, spinned down to avoid cell contamination and frozen until use.

Co-culture assay
Co-culturing of $CD34^+$ cells and HUVECs was performed in 24-well-plates with trans-well inserts (BD Biosciences). HUVECs were prior seeded with $2,5*10^4$ cells and grown until confluence. $7*10^4$ $CD34^+$ cells from Buffy Coats were transfected as described previously and washed twice with PBS before loading in 4µm trans-well inserts, which were already inserted in 24-well-plates. These $CD34^+$ cells and HUVECs were co-cultured for 48 h in EGM2. Afterwards, supernatant and HUVECs were collected and subsequent Real-Time PCR was performed.

***In-vivo* matrigel plug assay**
Animal experiments were approved by the local committee. Male NRMI nu/nu mice, aged 10-16 weeks were used for transplantation of human MNC sub populations. Mice were housed under specific pathogen-reduced conditions receiving autoclaved chow and water ad libitum.

In-vivo matrigel plug assay was performed as described previously(5) with the following modifications: $CD34^+$ cell fraction from Buffy Coats transfected with anti-miR, miR-mimic or scramble miR ($1x10^5$) were re-suspended in 100 µL of Matrigel Basement Membrane Matrix (BD Biosciences) containing 15 U of heparin (Sigma-Aldrich) and injected subcutaneously into 10 to 12 week-old male athymic nude mice along the abdominal midline. After 5 days, blood vessel infiltration in Matrigel plugs was quantified by analysis of CD31-staining (BD pharmingen) with FITC-anti-rat antibody (AbD Serotec). Sections were photographed with an inverted microscope (Olympus) at 10x magnification.

Chicken egg vascularization assay

The *in-vivo* angiogenesis assay was performed as described in detail previously,(6) using an assay allowing analysis of angiogenic properties of low numbers of $CD34^+$ progenitor cells. In brief, fertilized chicken eggs were incubated 3 days at 37°C before moving them in petri dishes (Corning Tissue Culture Dishes, Polystyrene, Sterile 100x20). After 7 further days of incubation, 100µl EGM-2 medium, scramble-miR or miR-mimic-378 transfected $CD34^+$ cells from Buffy Coats were resuspended in 100µl EGM-2 medium and implanted using silicone rings with 15mm diameter. Pictures of the chorioallantoic membrane (CAM) and embryo were taken after 0 and 48 hours with Leica M205 FA and Canon EOS 5D. Differences of vessel length within the chorioallantoic membrane between these time points were determined.

Statistical analysis

Data are presented as mean±SEM. Shapiro-Wilk and Kolmogorov-Smirnov tests were used to test for non-Gaussian distribution. Differences between groups were analysed by student t-test for two groups or by one-way ANOVA and followed by Dunnett T3 or Bonferroni´s multiple comparison test as a post hoc tests for normal distribution values. For variables without normal distribution Mann-Whitney U test or Kruskal-Wallis were used. Fisher´s exact test was used for comparison of qualitative data. All tests were performed two sided and a *p*-value of less than 0.05 was considered as statistically significant. Statistical analyses were performed using SPSS (version 21.0, SPSS Inc.) and GraphPad Prism 5.

References

1. Thygesen K, Alpert JS, White HD, Joint ESC/ACCF/AHA/WHF Task Force for the Redefinition of Myocardial Infarction. Universal definition of myocardial infarction. Eur Heart J. 2007 Oct;28(20):2525-2538.

2. Wu MH, Smith SL, Danet GH, Lin AM, Williams SF, Liebowitz DN, Dolan ME. Optimization of culture conditions to enhance transfection of human CD34+ cells by electroporation. Bone Marrow Transplant. 2001 Jun;27(11):1201-1209.

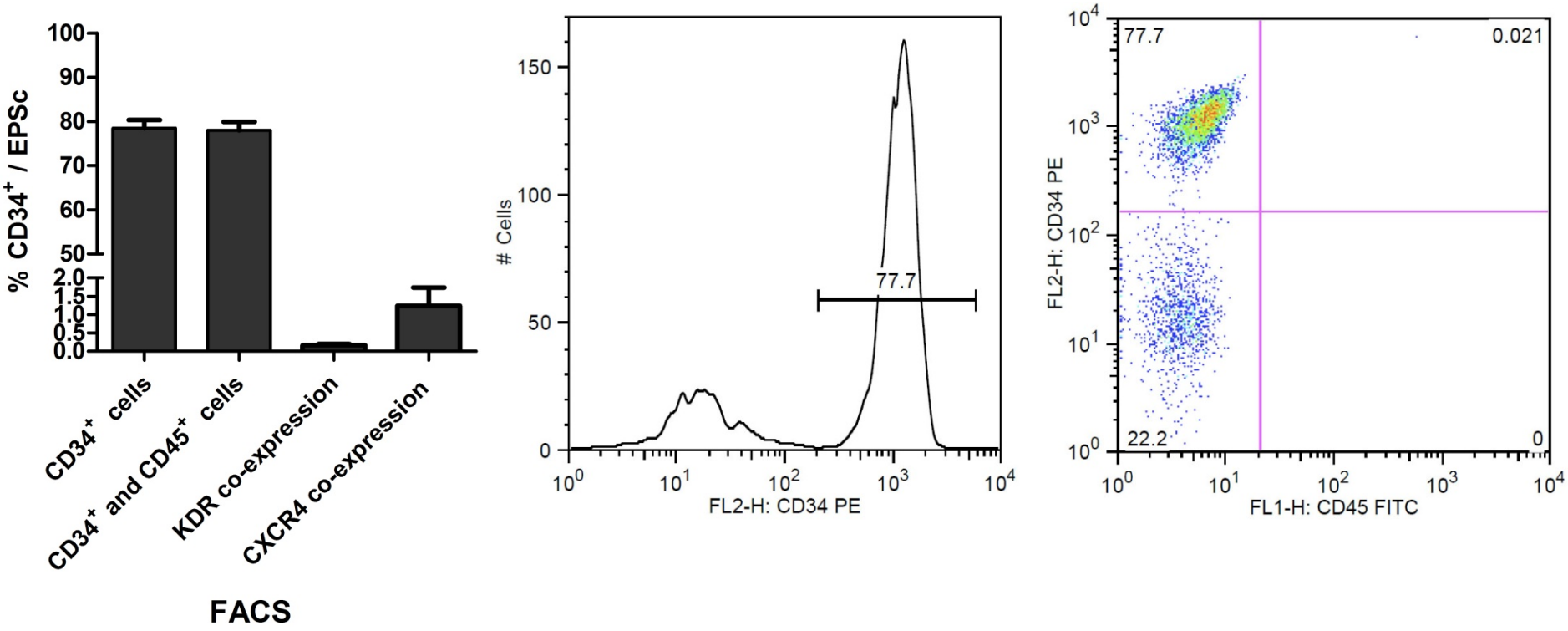
3. Templin C, Grote K, Schledzewski K, Ghadri JR, Schnabel S, Napp LC, Schieffer B, Kurzen H, Goerdt S, Landmesser U, Koenen W, Faulhaber J. Ex vivo expanded haematopoietic progenitor cells improve dermal wound healing by paracrine mechanisms. Exp Dermatol. 2009 May;18(5):445-453.

4. Menon MB, Ronkina N, Schwermann J, Kotlyarov A, Gaestel M. Fluorescence-based quantitative scratch wound healing assay demonstrating the role of MAPKAPK-2/3 in fibroblast migration. Cell Motil Cytoskeleton. 2009 Dec;66(12):1041-1047.

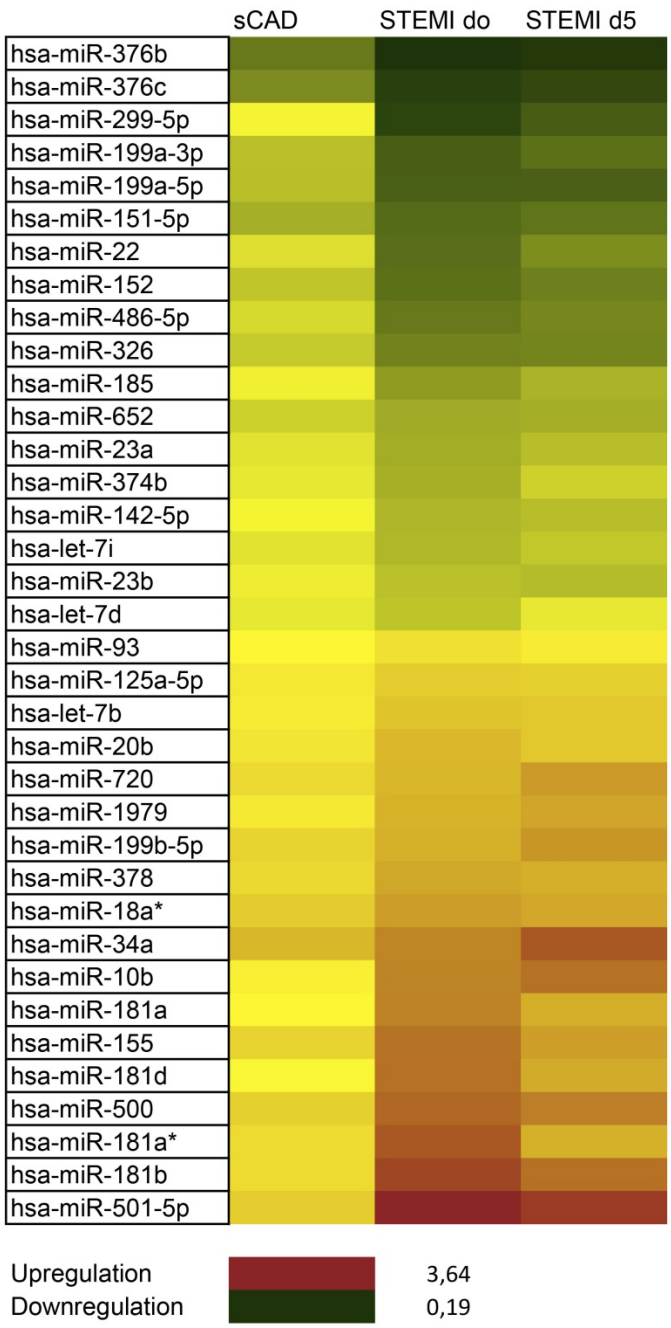
5. Mocharla P, Briand S, Giannotti G, Dorries C, Jakob P, Paneni F, Luscher T, Landmesser U. AngiomiR-126 expression and secretion from circulating CD34(+) and CD14(+) PBMCs: Role for proangiogenic effects and alterations in type 2 diabetics. Blood. 2013 Jan 3;121(1):226-236.

6. Djonov V, Schmid M, Tschanz SA, Burri PH. Intussusceptive angiogenesis: Its role in embryonic vascular network formation. Circ Res. 2000 Feb 18;86(3):286-292.

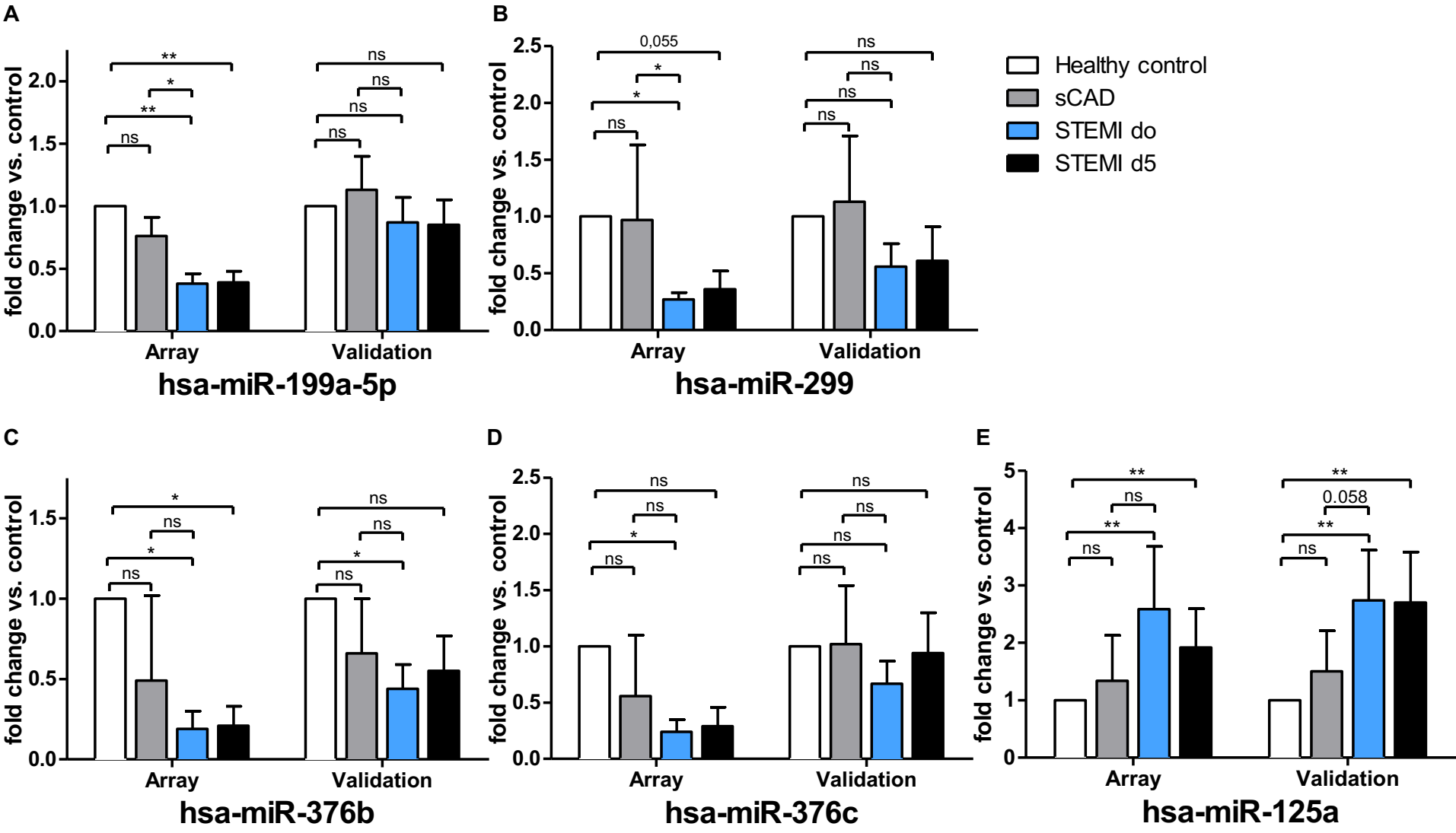
Supplemental material



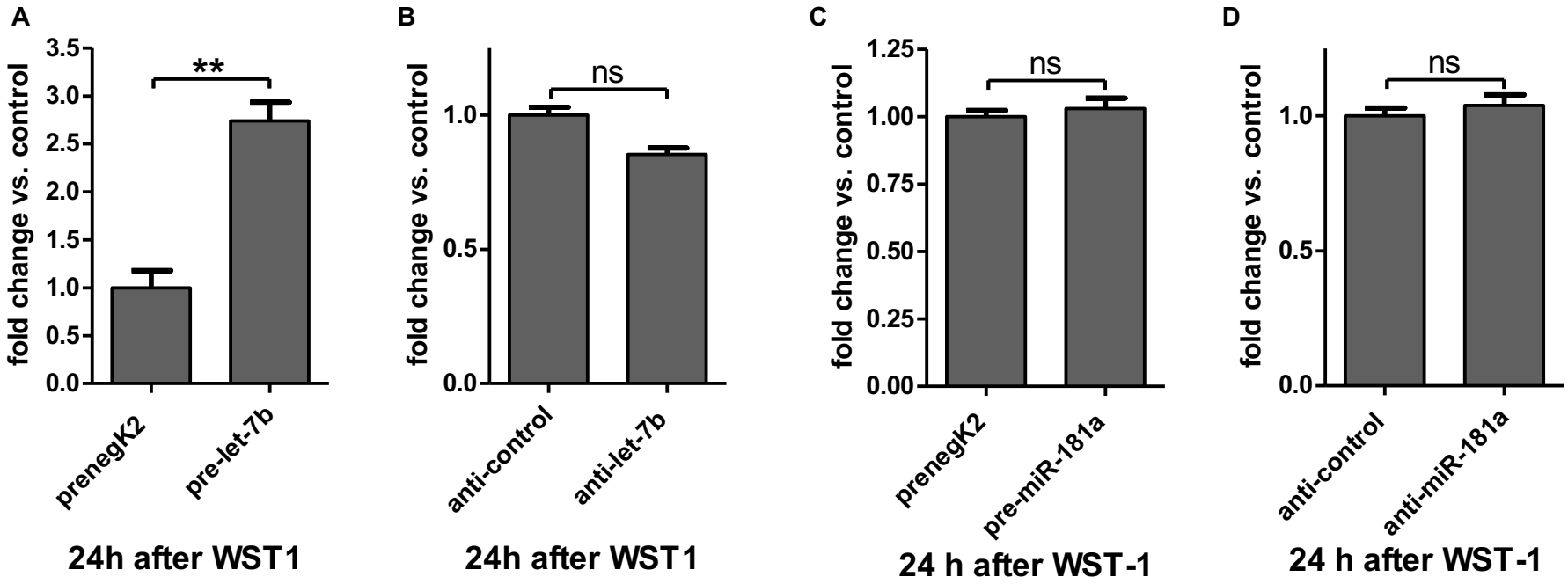
Supplemental Figure I. FACS results after $CD34^+$ cells isolation with anti-CD34 magnetic beads using two successive separations over LS MACS columns.



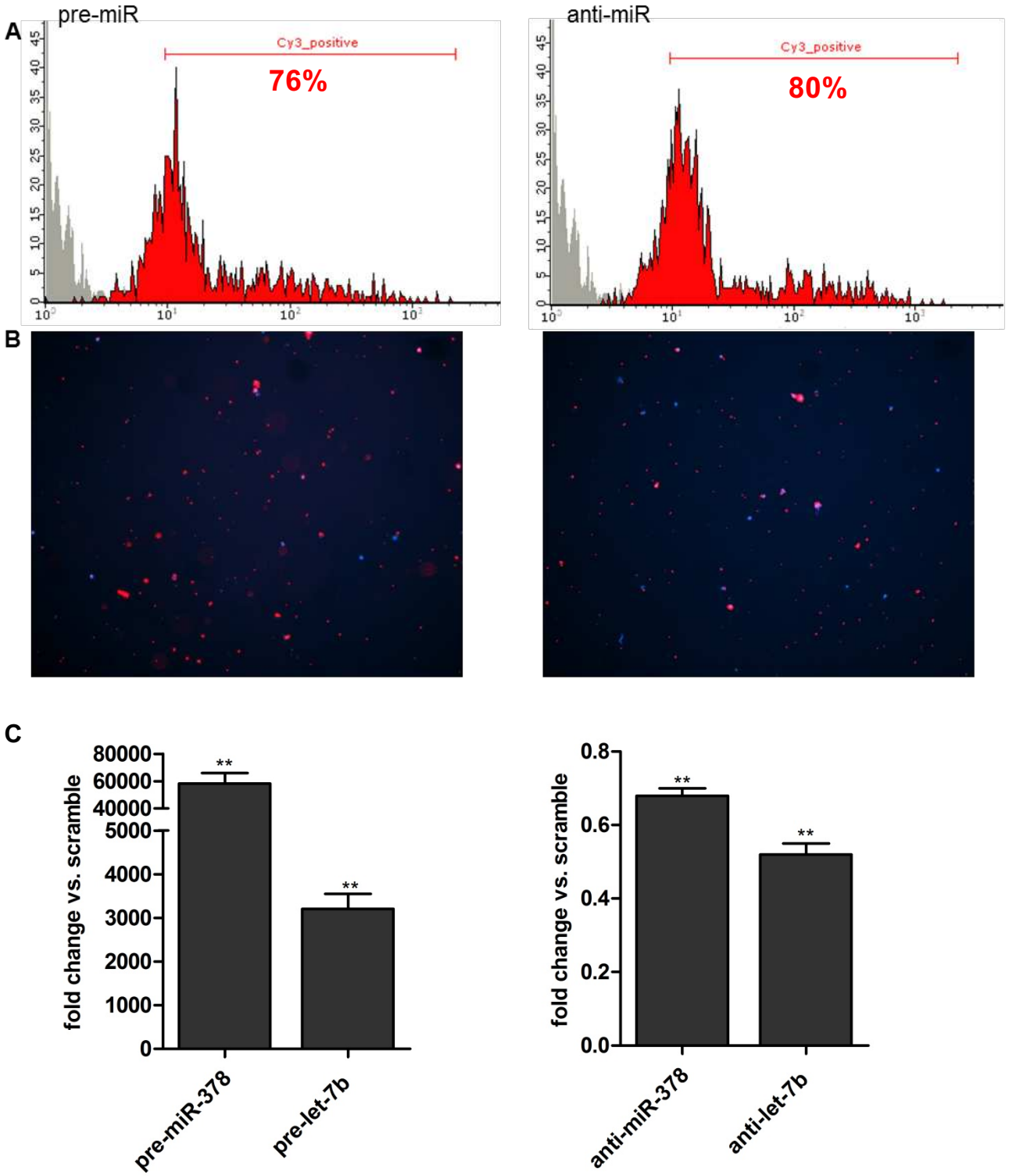
Supplemental Figure II. Heat Map of all microRNAs with a significant difference between healthy subjects and STEMI d0 ($p \leq 0.05$) in the miR-Array of $CD34^+$ progenitor cells. The highest fold change to healthy subjects over 0 is labeled in green and below 0 in red.



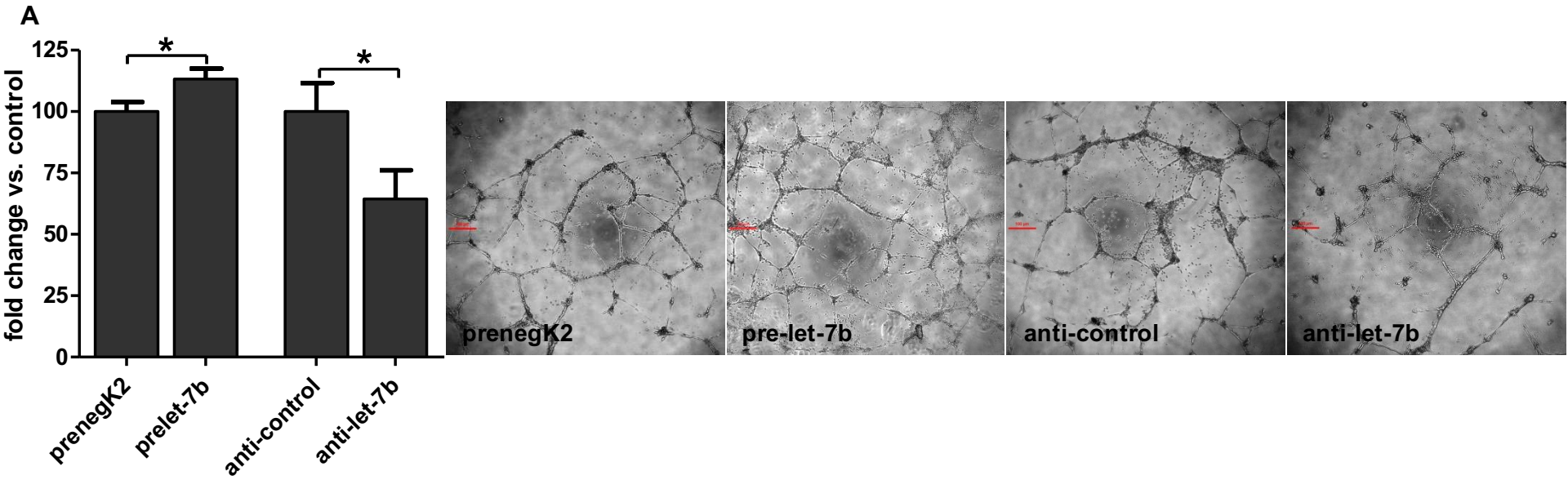
Supplemental Figure III. Results of the miRNA profiling and validation with Real-Time PCR. Micro-RNA expression levels for the miRNAs: **A,** miR-199a-5p**, B,** miR-299 , **C,** miR-376b, **D,** miR-376c, **E,** miR-125a in $CD34^{+}$ cells, isolated from study subjects. *=p<0.05, **=p<0.01, data are mean ± SEM

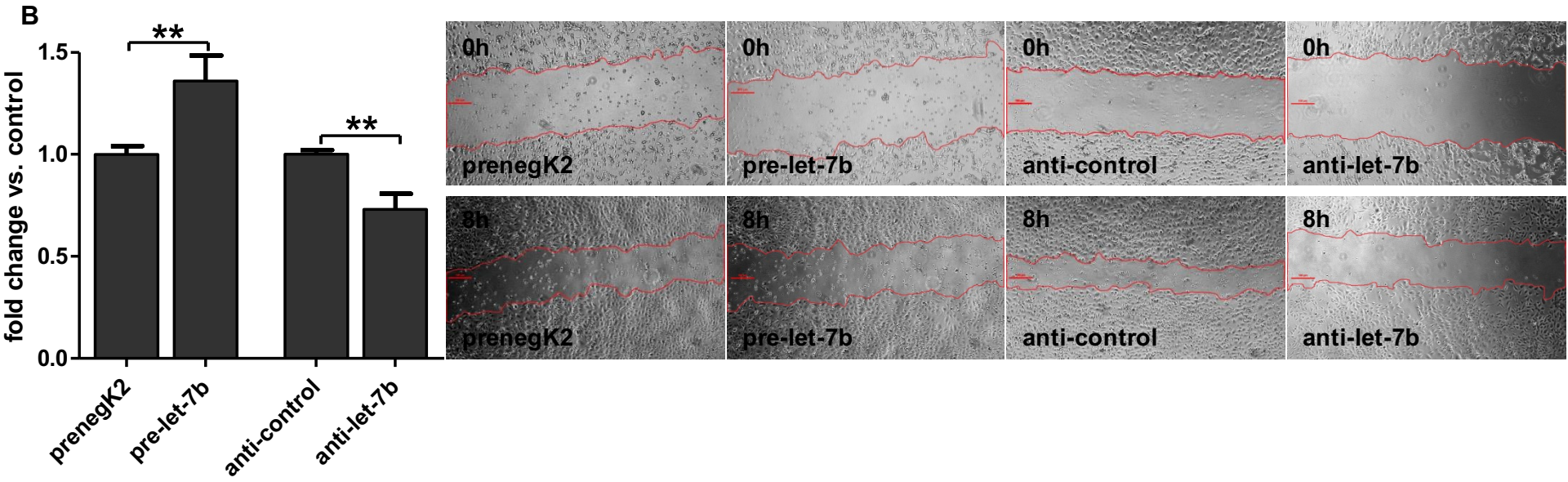


Supplemental Figure IV. Proliferation assay. Proliferation of $CD34^+$ progenitor cells ($1*10^4$) transfected with **A,** pre-let-7b, **B,** anti-let-7b, **C,** pre-181a and **D,** anti-miR-181a, quantified with WST1 proliferation reagent. *=$p<0.05$, data are mean ± SEM

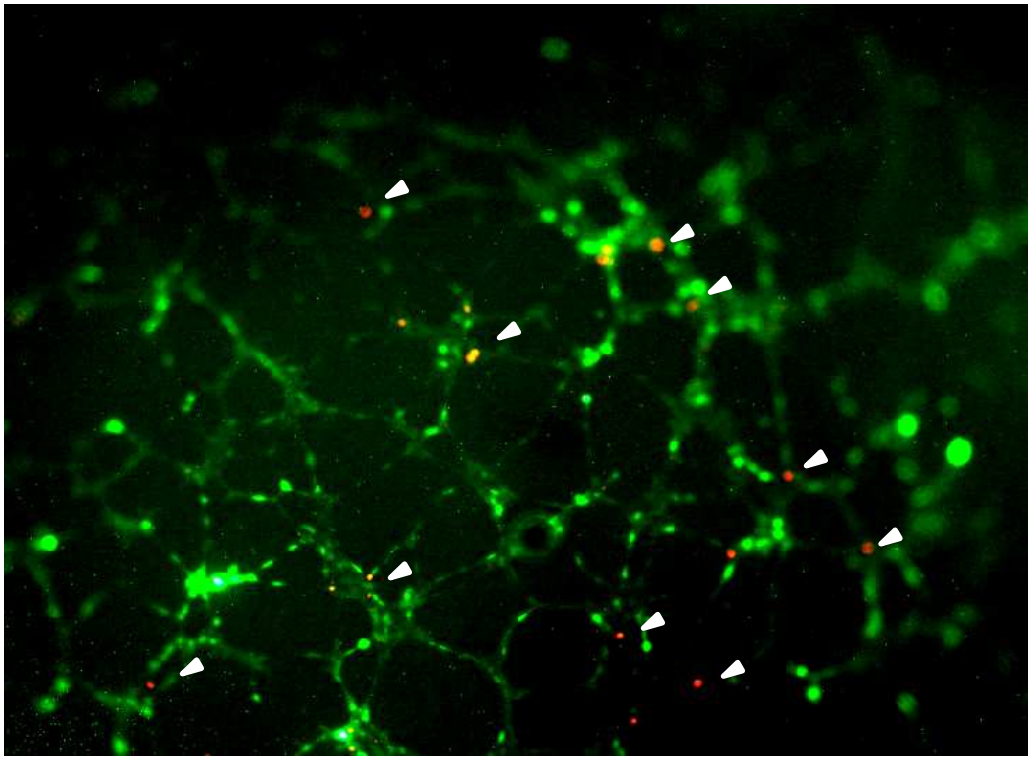


Supplemental Figure V. Transfection efficiency. **A,** FACS results 4h after transfection of the CD34⁺ cells with Cy-3 labeled pre-miR and anti-miR negative control. **B,** DAPI labeled CD34⁺ cells 4h after transfection with Cy-3 labeled pre-miR and anti-miR negative control (red). **C,** Micro-RNA expression levels in CD34⁺ cells after transfection with pre-miR or anti-miR.

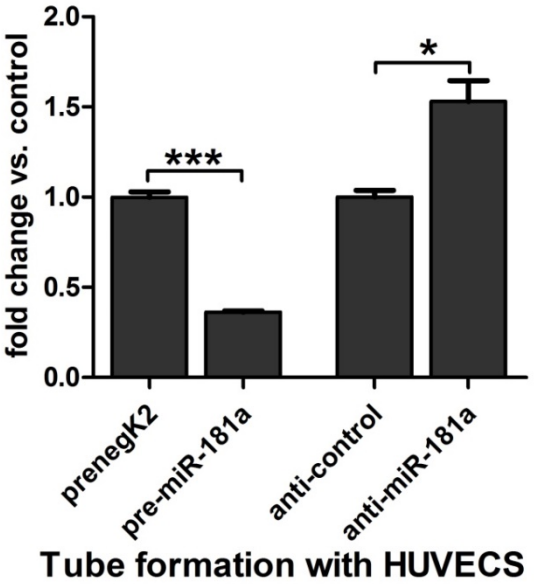


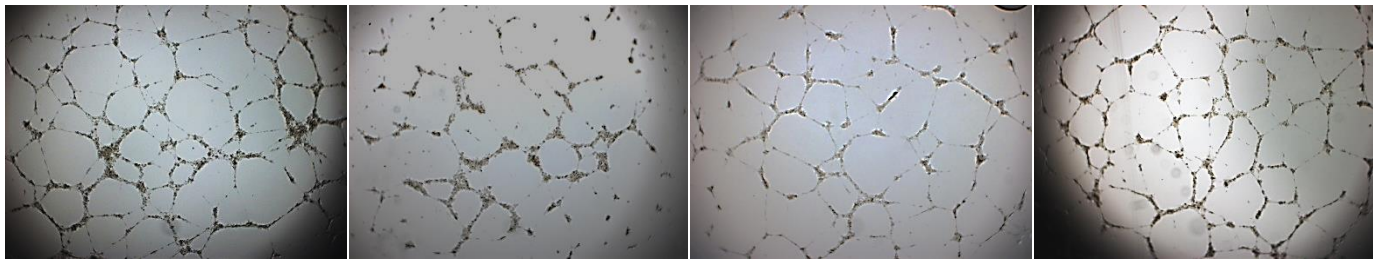


Supplemental Figure VI. *In-vitro* assays with scramble transfected CD34⁺ cells compared to pre-/anti-let-7b transfected CD34⁺ cells. **A,** Tube Formation angiogenesis assay with HUVECs ($1x10^4$) co-cultered for 18h with CD34⁺ transfected cells ($5x10^3$). Tube-like structures were quantified. **B,** Endothelial scratch migration assay. After scratching, HUVECs were cultured in starvation medium with scramble/miR-mimic-/anti-let-7b transfected CD34⁺ cells ($4x10^4$). The stimulatory effect of transfected CD34⁺ cells on endothelial cell migration capacity was measured using the migration index (8 hours after scratch). *=$p<0.05$, **=$p<0.01$, data are mean ± SEM

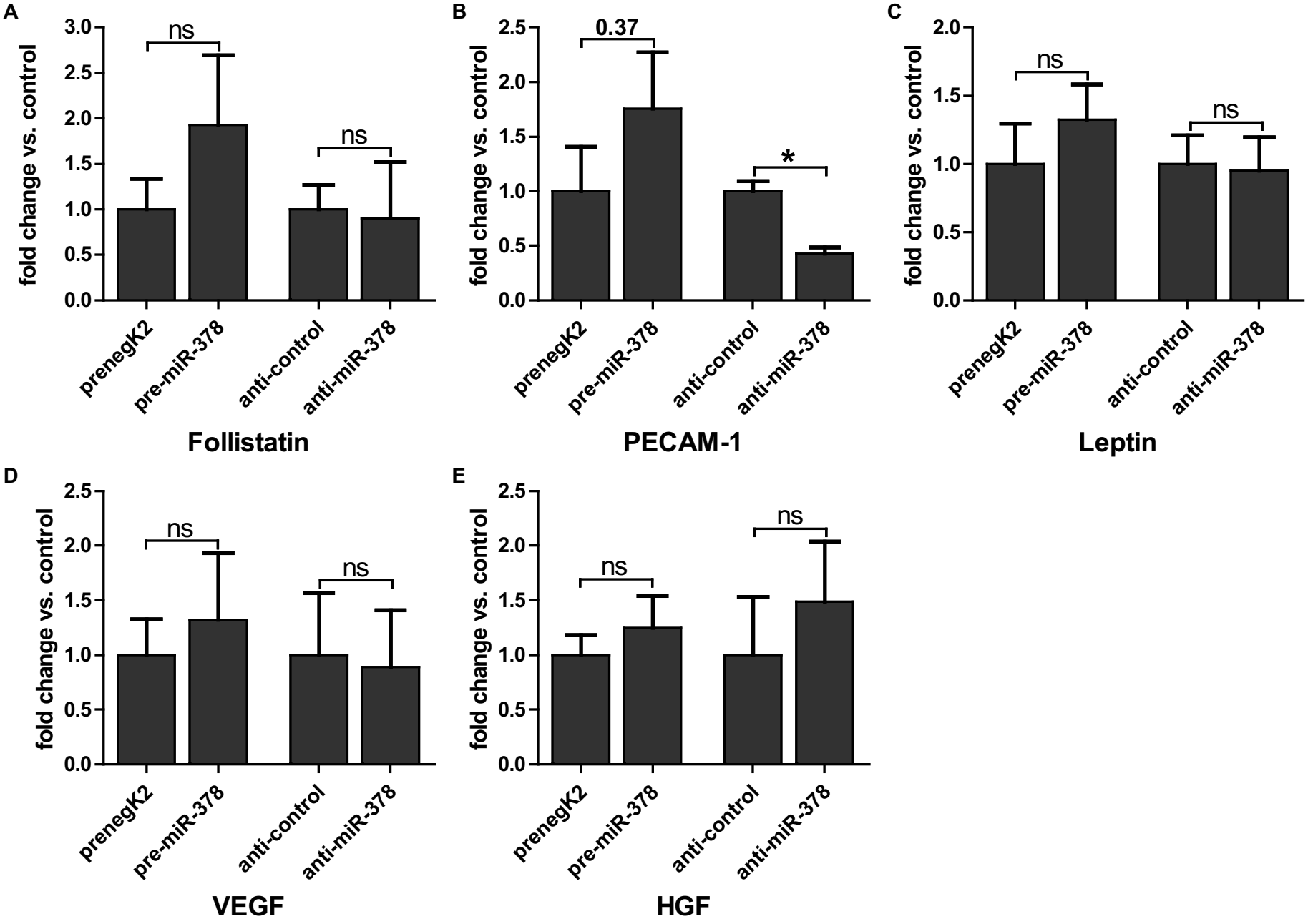


Supplemental Figure VII. Tube formation matrigel *in-vitro* assay with HUVECs (green) and $CD34^{+}$ cells (red).

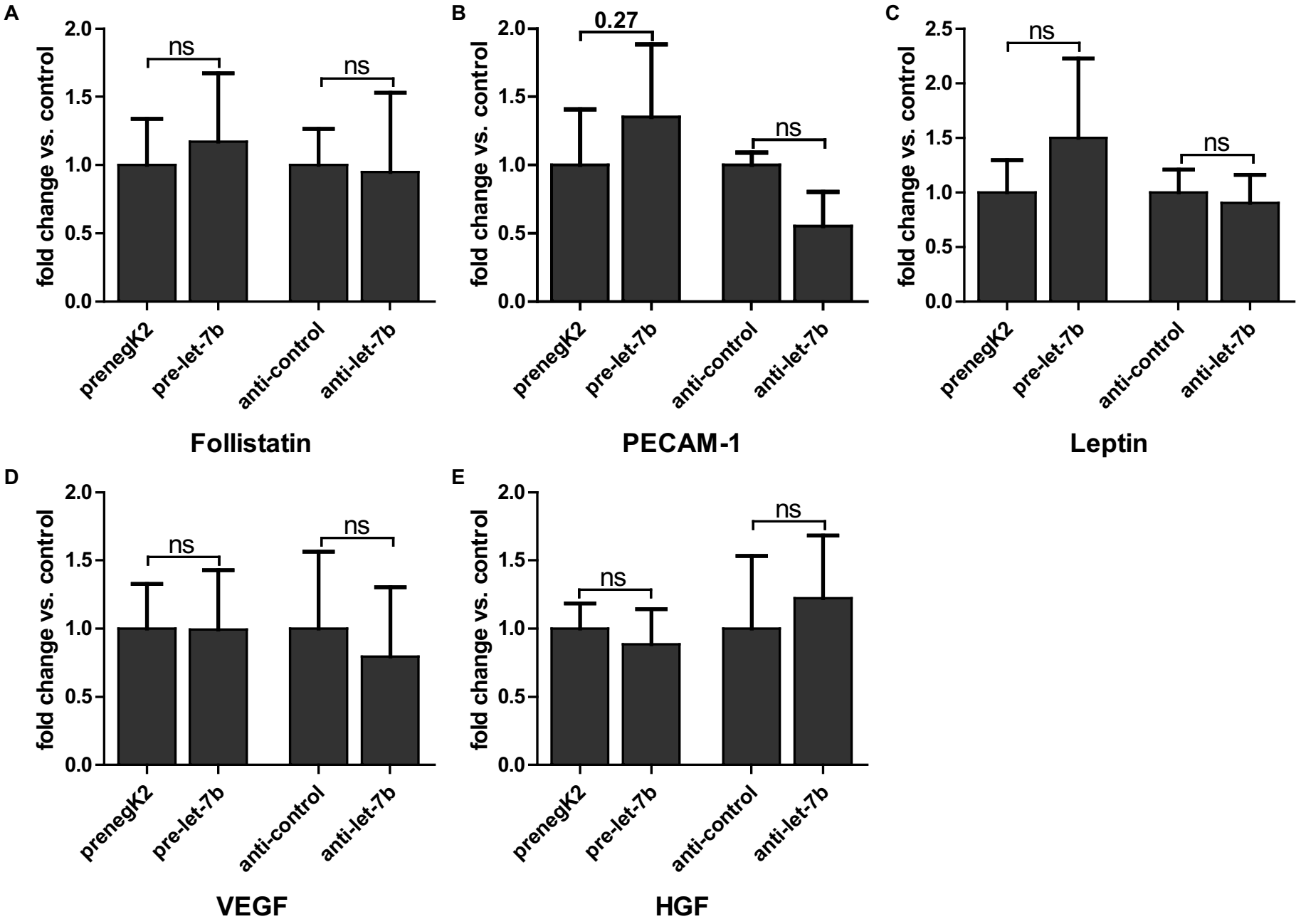




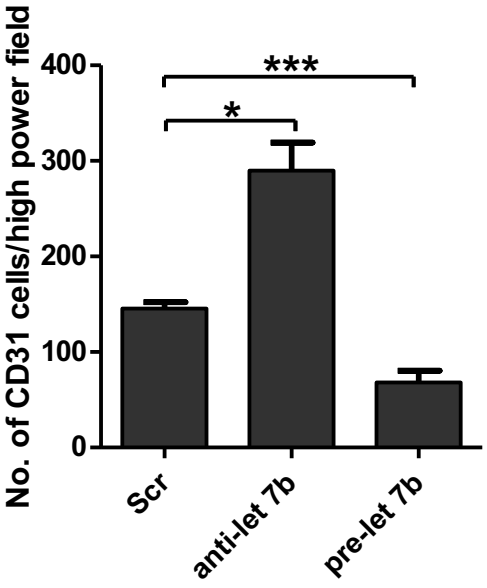
Supplemental Figure VIII. *In-vitro* assays with scramble transfected HUVECs compared to pre-/anti-miR-181a transfected HUVECS. Tube Formation angiogenesis assay with transfected HUVECs ($1x10^4$. Tube-like structures were quantified.

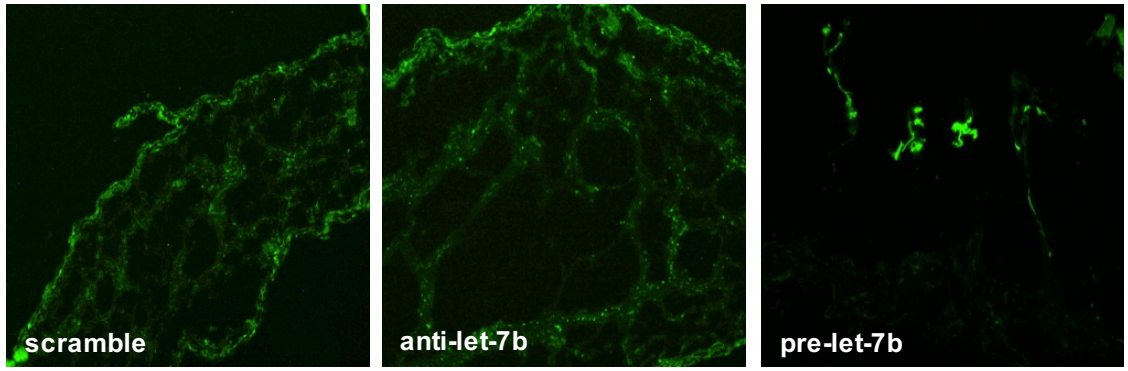


Supplemental Figure IXa. Cytokine assay with supernatant from CD34^{+} scramble/pre-/anti-miR-378 transfected cells for the following cytokines: **A,** Follistatin **B,** PECAM-1 **C,** Leptin **D,** VEGF **E,** HGF. Data are mean ± SEM



Supplemental Figure IXb. Cytokine assay with supernatant from CD34^{+} scramble/pre-/anti-let-7b transfected cells for the following cytokines: **A,** Follistatin **B,** PECAM-1 **C,** Leptin **D,** VEGF **E,** HGF. Data are mean ± SEM





***In-vivo* matrigel plug assay**

Supplemental Figure X. *In-vivo* matrigel plug assay. Blood vessel infiltration in Matrigel plugs containing ($1x10^5$) scramble/anti-/pre-miR-let-7b transfected $CD34^+$ cells was quantified by analysis of CD31-staining 5 days after injection. *=p<0.05, ***=p<0.001, data are mean ± SEM

Supplemental Table I. MiRNA-array for $CD34^+$ cells derived from healthy subjects, sCAD, STEMI d0 and STEMI d5 patients. The geomean of all assays detected in all samples was used for normalizing the data. Normalized data are Cp mean ± SEM, ranked by p-value between healthy subjects and STEMI d0. Difference in normalized Cps (ΔΔCp) between healthy subjects and STEMI d0 has been calculated. A fold change > 1 indicate up-regulation in STEMI d0 samples and a fold change < 1 indicate down-regulation in STEMI d0 samples compared to healthy control samples. Fold change = $2^{\Delta\Delta Cp}$. Only miRNAs measureable in all patients are provided.

Upregulated miRNAs in STEMI d0 samples compared to healthy subjects samples

miR-name	Healthy subjects	SEM	sCAD	SEM	STEMI d0	SEM	STEMI d5	SEM	p-value	Fold change
hsa-miR-1979	3,44	0,09	3,69	0,24	4,28	0,10	4,38	0,15	0,0008	1,79
hsa-miR-199b-5p	-3,53	0,14	-3,03	0,10	-2,68	0,08	-2,46	0,15	0,0018	1,81
hsa-miR-125a-5p	1,00	0,06	1,29	0,16	1,59	0,10	1,54	0,10	0,0021	1,50
hsa-miR-20b	-0,24	0,14	0,09	0,23	0,55	0,10	0,39	0,20	0,0038	1,73
hsa-miR-378	-1,65	0,16	-1,16	0,35	-0,71	0,13	-0,79	0,20	0,0040	1,91
hsa-miR-93	2,64	0,05	2,75	0,14	3,02	0,07	2,88	0,07	0,0062	1,30
hsa-miR-181a	2,98	0,28	3,09	0,40	4,20	0,16	3,85	0,18	0,0094	2,32
hsa-miR-17	3,77	0,07	3,96	0,19	4,16	0,08	4,07	0,13	0,0095	1,32
hsa-miR-424	0,04	0,19	0,38	0,38	1,05	0,19	0,64	0,27	0,0098	2,02
hsa-miR-19b	5,23	0,08	5,46	0,28	5,80	0,13	5,66	0,14	0,0108	1,48
hsa-miR-181b	-1,92	0,38	-1,48	0,45	-0,31	0,23	-0,60	0,25	0,0116	3,05
hsa-miR-106a	3,68	0,07	3,88	0,19	4,12	0,10	4,01	0,11	0,0116	1,35
hsa-miR-155	0,05	0,33	0,55	0,34	1,36	0,15	1,05	0,16	0,0116	2,47
hsa-let-7b	0,74	0,11	0,97	0,18	1,42	0,16	1,38	0,15	0,0130	1,61
hsa-miR-181d	-0,75	0,28	-0,78	0,42	0,57	0,26	0,15	0,23	0,0136	2,49
hsa-miR-501-5p	-7,11	0,37	-6,53	0,34	-5,25	0,40	-5,42	0,38	0,0137	3,64
hsa-miR-21*	-5,47	0,11	-4,90	0,38	-4,66	0,21	-5,15	0,37	0,0139	1,75
hsa-miR-92a-1*	-5,09	0,46	-4,62	0,61	-3,36	0,27	-4,09	0,43	0,0172	3,32
hsa-miR-34a	-3,73	0,21	-2,93	0,31	-2,55	0,31	-2,24	0,36	0,0195	2,26
hsa-miR-18a*	-3,62	0,19	-3,03	0,21	-2,62	0,26	-2,69	0,19	0,0201	2,01
hsa-miR-92a	4,07	0,09	4,26	0,28	4,75	0,20	4,64	0,17	0,0219	1,60
hsa-miR-20a	4,48	0,06	4,69	0,22	4,99	0,14	4,90	0,14	0,0242	1,43
hsa-miR-99a*	-5,50	0,29	-5,84	0,61	-4,52	0,18	-5,55	0,36	0,0277	1,97
hsa-miR-106b	0,94	0,06	0,98	0,13	1,19	0,07	1,13	0,11	0,0311	1,19
hsa-miR-582-5p	-4,30	0,37	-4,44	0,51	-2,95	0,32	-3,11	0,40	0,0335	2,55
hsa-miR-125b	-0,27	0,34	0,15	0,57	1,11	0,37	0,68	0,27	0,0353	2,59
hsa-miR-196b	-0,23	0,26	-0,13	0,40	0,79	0,29	0,65	0,35	0,0370	2,03
hsa-miR-500	-5,58	0,39	-5,03	0,37	-4,21	0,35	-4,36	0,28	0,0385	2,60
hsa-miR-197	-0,23	0,09	-0,08	0,17	0,18	0,13	0,12	0,14	0,0414	1,33
hsa-miR-140-5p	-1,35	0,10	-1,29	0,13	-0,98	0,11	-1,05	0,17	0,0437	1,30
hsa-miR-10b	-3,01	0,41	-2,84	0,33	-1,82	0,24	-1,69	0,39	0,0450	2,28
hsa-miR-25	-0,15	0,06	-0,06	0,20	0,27	0,14	0,32	0,11	0,0510	1,34
hsa-miR-181c	-2,93	0,19	-2,84	0,42	-2,20	0,25	-2,18	0,29	0,0547	1,66
hsa-miR-181a*	-4,06	0,50	-3,62	0,39	-2,57	0,38	-3,20	0,21	0,0552	2,80
hsa-miR-222	1,61	0,22	1,87	0,39	2,33	0,21	2,10	0,22	0,0570	1,65
hsa-let-7g	3,16	0,08	3,25	0,12	3,37	0,04	3,17	0,04	0,0581	1,16
hsa-miR-19a	4,53	0,05	4,66	0,19	4,90	0,15	4,80	0,14	0,0588	1,29
hsa-miR-365	-4,60	0,40	-4,44	0,50	-3,53	0,23	-3,41	0,24	0,0599	2,10

hsa-miR-99a	-0,93	0,35	-0,55	0,56	0,23	0,36	-0,05	0,27	0,0607	2,23
hsa-miR-720	2,74	0,13	3,19	0,29	3,54	0,29	3,77	0,22	0,0608	1,75
hsa-miR-363	-2,64	0,16	-2,33	0,34	-1,98	0,25	-1,85	0,18	0,0655	1,59
hsa-miR-629	-5,60	0,50	-4,81	0,25	-4,31	0,33	-4,26	0,26	0,0769	2,44
hsa-miR-324-3p	-1,50	0,14	-1,23	0,12	-1,15	0,10	-1,35	0,10	0,0827	1,27
hsa-miR-92b	-4,90	0,27	-4,32	0,27	-3,97	0,39	-4,12	0,35	0,0962	1,90
hsa-miR-196a	-5,69	0,51	-6,21	0,46	-4,52	0,22	-4,97	0,20	0,1015	2,26
hsa-miR-10a	-0,70	0,37	-0,45	0,54	0,34	0,43	0,29	0,36	0,1143	2,06
hsa-miR-532-5p	-4,03	0,25	-3,94	0,26	-3,48	0,17	-3,55	0,25	0,1186	1,46
hsa-miR-146b-5p	-2,36	0,32	-1,92	0,14	-1,48	0,37	-1,45	0,17	0,1201	1,85
hsa-miR-651	-6,61	0,37	-6,76	0,13	-5,91	0,20	-6,29	0,20	0,1491	1,62
hsa-miR-15a	4,00	0,10	4,20	0,12	4,25	0,12	4,12	0,12	0,1655	1,19
hsa-miR-421	-4,44	0,19	-4,18	0,20	-4,09	0,16	-4,15	0,15	0,2025	1,27
hsa-miR-140-3p	0,25	0,11	0,20	0,14	0,45	0,12	0,48	0,12	0,2566	1,16
hsa-miR-660	-5,02	0,11	-5,14	0,46	-4,83	0,11	-4,61	0,37	0,2697	1,14
hsa-miR-192	-3,65	0,08	-3,78	0,16	-3,26	0,32	-3,33	0,17	0,3168	1,31
hsa-miR-450a	-4,52	0,27	-4,55	0,30	-4,01	0,42	-4,20	0,41	0,3422	1,43
hsa-let-7c	1,30	0,04	1,23	0,13	1,44	0,12	1,60	0,08	0,3514	1,10
hsa-miR-32	-4,98	0,29	-4,65	0,38	-4,60	0,25	-5,25	0,12	0,3566	1,30
hsa-miR-7	-6,82	0,48	-6,12	0,39	-6,30	0,22	-6,08	0,19	0,3766	1,44
hsa-miR-16	4,39	0,19	4,61	0,17	4,59	0,09	4,58	0,13	0,3846	1,15
hsa-miR-29b-2*	-4,39	0,13	-4,48	0,24	-4,02	0,36	-4,88	0,13	0,3896	1,29
hsa-miR-338-3p	-0,36	0,12	-0,20	0,10	-0,17	0,18	-0,28	0,18	0,4235	1,14
hsa-miR-200a	-7,40	0,17	-6,80	0,54	-6,99	0,46	-6,31	0,49	0,4289	1,33
hsa-miR-423-3p	1,86	0,16	1,97	0,10	2,00	0,05	1,91	0,04	0,4369	1,10
hsa-miR-361-3p	-2,47	0,18	-2,37	0,16	-2,24	0,23	-2,44	0,25	0,4653	1,17
hsa-miR-194	-5,48	0,11	-5,42	0,13	-5,36	0,16	-5,27	0,07	0,5381	1,09
hsa-miR-30e*	-1,46	0,06	-1,60	0,11	-1,42	0,04	-1,47	0,09	0,5902	1,03
hsa-miR-423-5p	-0,88	0,07	-0,88	0,05	-0,82	0,09	-0,97	0,09	0,6030	1,04
hsa-miR-296-5p	-4,55	0,38	-4,71	0,35	-4,23	0,50	-4,37	0,34	0,6294	1,25
hsa-miR-215	-5,53	0,14	-5,52	0,08	-5,40	0,22	-5,66	0,14	0,6334	1,10
hsa-miR-320a	2,01	0,14	2,20	0,10	2,12	0,19	2,16	0,09	0,6373	1,08
hsa-miR-505	-2,90	0,18	-2,74	0,05	-2,81	0,06	-2,57	0,09	0,6472	1,07
hsa-miR-342-3p	1,00	0,23	0,88	0,25	1,15	0,21	1,00	0,07	0,6502	1,11
hsa-miR-301b	-4,02	0,28	-4,27	0,21	-3,86	0,25	-4,19	0,16	0,6964	1,11
hsa-miR-30b	3,78	0,10	3,73	0,13	3,81	0,06	3,72	0,03	0,8064	1,02
hsa-miR-186	0,22	0,03	0,21	0,08	0,24	0,11	0,26	0,08	0,8084	1,02
hsa-miR-30e	2,03	0,08	1,83	0,13	2,06	0,10	1,90	0,10	0,8242	1,02
hsa-miR-135a	-7,16	0,34	-7,26	0,16	-7,08	0,21	-6,68	0,30	0,8427	1,06
hsa-let-7d*	-0,67	0,15	-0,55	0,09	-0,67	0,06	-0,55	0,05	0,9743	1,00
hsa-miR-195	-3,66	0,41	-3,90	0,31	-3,64	0,18	-3,78	0,12	0,9797	1,01
hsa-let-7f	3,40	0,07	3,26	0,08	3,40	0,04	3,29	0,01	0,9853	1,00
hsa-let-7a	3,89	0,18	3,78	0,20	3,89	0,61	4,07	0,13	0,9950	1,00
hsa-miR-142-3p	6,82	0,11	6,79	0,14	6,83	0,07	6,78	0,10	0,9963	1,00

Downregulated miRNAs in STEMI d0 samples compared to healthy subjects samples										
miR-name	**Healthy subjects**	**SEM**	**sCAD**	**SEM**	**STEMI d0**	**SEM**	**STEMI d5**	**SEM**	**p-value**	**Fold change**
hsa-miR-299-5p	-4,55	0,26	-4,60	0,70	-6,46	0,17	-6,00	0,45	,001	0,27
hsa-let-7i	1,02	0,05	0,88	0,08	0,58	0,03	0,69	0,13	,001	0,74
hsa-miR-374b	0,11	0,08	-0,02	0,15	-0,40	0,03	-0,14	0,03	,001	0,71
hsa-miR-487b	-5,24	0,33	-6,24	1,00	-7,34	0,22	-6,98	0,46	,002	0,23
hsa-miR-199a-5p	1,80	0,10	1,41	0,24	0,42	0,25	0,43	0,28	,002	0,38
hsa-miR-151-5p	2,10	0,09	1,59	0,27	0,87	0,23	0,99	0,22	,002	0,43
hsa-miR-199a-3p	0,68	0,19	0,30	0,28	-0,72	0,23	-0,47	0,28	,003	0,38
hsa-miR-152	-1,68	0,24	-2,01	0,20	-2,83	0,03	-2,64	0,20	,003	0,45
hsa-miR-486-5p	-0,91	0,04	-1,12	0,39	-1,94	0,16	-1,79	0,34	,006	0,49
hsa-miR-376c	-1,50	0,39	-2,34	0,89	-3,54	0,38	-3,30	0,56	,010	0,24
hsa-miR-376b	-2,84	0,33	-3,87	1,01	-5,24	0,57	-5,09	0,57	,011	0,19
hsa-miR-326	-0,67	0,11	-0,97	0,17	-1,60	0,24	-1,57	0,13	,013	0,53
hsa-miR-185	1,78	0,05	1,70	0,21	1,10	0,14	1,30	0,15	,013	0,63
hsa-miR-376a	-1,11	0,43	-2,14	1,02	-3,18	0,44	-2,87	0,56	,015	0,24
hsa-miR-411	-3,64	0,33	-4,39	0,94	-5,62	0,49	-5,68	0,77	,016	0,25
hsa-miR-22	-1,13	0,22	-1,30	0,21	-2,31	0,28	-1,97	0,25	,017	0,44
hsa-miR-154	-2,58	0,46	-3,51	0,79	-4,76	0,49	-4,29	0,64	,018	0,22
hsa-miR-23b	4,21	0,10	4,12	0,11	3,84	0,06	3,80	0,06	,020	0,77
hsa-miR-221	2,80	0,09	2,73	0,15	2,38	0,10	2,34	0,14	,022	0,75
hsa-let-7d	-0,06	0,09	-0,19	0,08	-0,41	0,07	-0,18	0,08	,022	0,79
hsa-miR-142-5p	1,93	0,08	1,88	0,05	1,49	0,12	1,53	0,10	,023	0,74
hsa-miR-127-3p	-2,55	0,16	-2,88	0,89	-4,10	0,50	-3,72	0,58	,025	0,34
hsa-miR-382	-2,78	0,42	-3,58	0,75	-4,69	0,49	-4,62	0,67	,025	0,27
hsa-miR-153	-4,93	0,17	-5,50	0,65	-5,91	0,29	-5,91	0,24	,027	0,51
hsa-miR-23a	4,61	0,12	4,47	0,06	4,09	0,14	4,22	0,08	,027	0,69
hsa-miR-200c	-3,91	0,13	-3,98	0,21	-4,45	0,14	-3,97	0,25	,033	0,69
hsa-miR-652	0,00	0,16	-0,25	0,14	-0,53	0,05	-0,52	0,16	,035	0,69
hsa-miR-584	-3,39	0,32	-3,85	0,46	-4,46	0,30	-4,25	0,44	,050	0,48
hsa-miR-133a	-2,08	0,26	-2,09	0,56	-3,01	0,28	-2,55	0,49	,052	0,52
hsa-miR-191	1,74	0,07	1,61	0,12	1,43	0,11	1,54	0,12	,054	0,80
hsa-miR-24	4,01	0,06	4,05	0,05	3,84	0,04	3,89	0,07	,061	0,89
hsa-miR-28-5p	-0,51	0,12	-0,53	0,12	-1,00	0,18	-0,86	0,05	,061	0,71
hsa-miR-103	4,73	0,15	4,59	0,07	4,35	0,08	4,33	0,09	,066	0,77
hsa-miR-328	-1,21	0,05	-1,33	0,16	-1,36	0,05	-1,48	0,07	,071	0,90
hsa-miR-330-3p	-4,74	0,27	-5,18	0,21	-5,40	0,13	-4,98	0,18	,075	0,64
hsa-miR-148b	0,92	0,14	0,70	0,04	0,37	0,21	0,31	0,16	,083	0,69
hsa-miR-145	-0,65	0,14	-0,59	0,49	-1,40	0,36	-1,37	0,35	,101	0,59
hsa-miR-21	4,48	0,11	4,53	0,29	4,01	0,23	4,40	0,46	,108	0,72
hsa-miR-148a	-1,70	0,36	-1,64	0,17	-2,58	0,31	-2,23	0,29	,111	0,54
hsa-miR-744	-3,84	0,26	-4,14	0,18	-4,42	0,06	-4,40	0,15	,112	0,67
hsa-miR-625*	-3,80	0,37	-4,22	0,49	-4,85	0,43	-4,50	0,29	,114	0,48
hsa-miR-126	4,84	0,12	4,82	0,21	4,59	0,06	4,61	0,11	,114	0,84

hsa-miR-22*	-2,24	0,17	-2,09	0,19	-2,98	0,37	-2,78	0,29	,117	0,60
hsa-miR-107	3,37	0,08	3,15	0,16	2,92	0,21	3,12	0,09	,121	0,73
hsa-miR-182	-5,23	0,39	-5,75	0,46	-6,39	0,52	-6,07	0,45	,124	0,45
hsa-miR-29c	0,80	0,16	0,61	0,18	0,42	0,16	0,57	0,09	,137	0,76
hsa-miR-484	1,63	0,14	1,58	0,14	1,28	0,15	1,34	0,12	,149	0,79
hsa-miR-18a	-0,84	0,10	-0,94	0,19	-1,09	0,12	-1,11	0,13	,156	0,84
hsa-miR-1	-3,14	0,35	-2,91	0,53	-4,26	0,60	-4,07	0,54	,158	0,46
hsa-miR-425	0,73	0,10	0,70	0,06	0,54	0,07	0,60	0,07	,164	0,88
hsa-miR-26b	1,47	0,12	1,38	0,21	1,26	0,05	1,27	0,16	,173	0,87
hsa-miR-143	-1,35	0,22	-1,57	0,44	-2,00	0,38	-2,05	0,33	,193	0,64
hsa-miR-628-3p	-4,36	0,20	-5,04	0,14	-5,08	0,43	-5,16	0,08	,196	0,61
hsa-miR-133b	-3,58	0,26	-3,39	0,42	-4,34	0,48	-3,95	0,48	,213	0,59
hsa-miR-139-5p	-1,33	0,16	-0,97	0,54	-1,68	0,21	-1,78	0,48	,217	0,78
hsa-miR-340	-3,73	0,30	-3,99	0,18	-4,86	0,88	-4,72	0,42	,273	0,46
hsa-miR-331-3p	-0,23	0,17	-0,26	0,15	-0,45	0,08	-0,60	0,09	,275	0,86
hsa-miR-339-5p	-0,73	0,14	-1,09	0,08	-0,95	0,12	-0,87	0,09	,292	0,86
hsa-miR-29b	-1,06	0,23	-1,13	0,16	-1,38	0,16	-1,75	0,53	,298	0,80
hsa-miR-301a	-1,31	0,24	-1,67	0,30	-1,76	0,32	-1,66	0,33	,306	0,73
hsa-miR-30d	1,06	0,20	0,63	0,13	0,79	0,13	0,87	0,19	,311	0,83
hsa-miR-590-5p	-0,62	0,14	-0,62	0,15	-0,79	0,07	-1,00	0,19	,314	0,89
hsa-miR-193b	-6,06	0,30	-6,71	0,27	-6,90	0,73	-5,35	0,27	,333	0,56
hsa-miR-30a	-1,47	0,29	-1,54	0,36	-1,93	0,35	-1,44	0,17	,352	0,73
hsa-miR-497	-4,39	0,33	-4,47	0,14	-4,89	0,38	-4,47	0,13	,359	0,71
hsa-miR-425*	-1,98	0,12	-2,19	0,14	-2,14	0,12	-2,29	0,12	,380	0,89
hsa-miR-150	2,75	0,32	2,14	0,27	2,34	0,33	2,46	0,19	,407	0,75
hsa-miR-141	-4,78	0,06	-4,73	0,22	-5,24	0,49	-5,10	0,26	,418	0,73
hsa-miR-130b	-3,97	0,12	-4,13	0,18	-4,09	0,12	-4,13	0,14	,514	0,92
hsa-miR-766	-1,06	0,06	-1,09	0,14	-1,15	0,13	-1,17	0,13	,521	0,94
hsa-miR-9	-6,15	0,19	-5,47	0,43	-6,63	0,66	-6,41	0,20	,531	0,72
hsa-miR-146a	-0,14	0,12	-0,12	0,20	-0,38	0,35	-0,46	0,15	,565	0,85
hsa-miR-130a	0,03	0,13	0,11	0,16	-0,07	0,14	-0,09	0,12	,600	0,93
hsa-miR-99b	-1,02	0,07	-1,12	0,22	-1,14	0,21	-1,38	0,26	,621	0,92
hsa-miR-98	-4,74	0,19	-4,61	0,10	-4,84	0,10	-4,97	0,18	,636	0,93
hsa-miR-503	-3,70	0,36	-3,52	0,49	-3,90	0,26	-3,75	0,25	,673	0,87
hsa-miR-29a	0,07	0,15	0,06	0,18	0,00	0,10	0,09	0,06	,694	0,95
hsa-miR-223	7,01	0,09	6,99	0,04	6,95	0,17	6,87	0,14	,751	0,96
hsa-miR-451	0,30	0,23	0,04	0,36	0,15	0,43	0,63	0,45	,771	0,90
hsa-let-7e	-1,38	0,22	-1,33	0,14	-1,43	0,14	-1,28	0,22	,841	0,96
hsa-miR-18b	0,03	0,14	0,14	0,13	0,01	0,08	-0,13	0,21	,899	0,99
hsa-miR-335	-0,96	0,10	-1,17	0,32	-0,99	0,27	-1,27	0,07	,919	0,98
hsa-miR-15b	3,20	0,09	3,20	0,07	3,19	0,04	3,12	0,04	,921	0,99